

CONGENITAL CYTOMEGALOVIRUS INFECTION.

A study of a British population.

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SUMMARY

In a prospective study, 50 children with congenital cytomegalovirus (CMV) infection were followed up for periods of one to three years. Thirty two infants were born following maternal primary infection, ten following maternal recurrent infection and the type of infection could not be defined in eight cases. Three (6%) were symptomatic in the neonatal period, two with neurological abnormalities, one with pneumonitis. In the first six months of life, two suffered transient hepatosplenomegaly, and six (12%) pneumonitis. Six (12%) suffered long-term sequelae, one spastic quadraplegia with optic atrophy, one spastic quadraplegia with sensorineural deafness and four sensorineural deafness alone. In three cases (6%) deafness was bilateral and hearing aids were required.

There was no evidence that congenital CMV caused mental retardation, intrauterine or postnatal growth retardation, in the absence of neurological abnormalities. Children with microcephaly or symptomatic in the neonatal period had a poor prognosis. No other indicator including type and timing of maternal infection, titre of CMV specific IgM in cord blood, persistence of viral excretion, or maternal socio-economic background was associated with poor outcome.

Women under twenty years, black women and single women were significantly more likely to give birth to a congenitally infected infant than white, married women over 25 years with a relative risk of 3.9, 2.1 and 2.5 respectively.

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Statement of originality:

This work has been performed by me with others whose contribution is gratefully acknowledged above.

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LIST OF ABBREVIATIONS.

CF	Complement fixation.
CID	Cytomegalic inclusion disease.
CMI	Cell mediated immunity
CMV	Cytomegalovirus
CNS	Central nervous system
CPE	Cytopathic effect
CT	Computerised tomography
DNA	Deoxyribose nucleic acid
ELISA	Enzyme-linked immunosorbant assay
IF	Immunofluorescent
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LMP	Last menstrual period
LTR	Lymphocyte transformation (proliferation) response
MACRIA	Macroglobulin antibody capture radioimmune assay
OFC	Occipital frontal circumference
PHA	Phytohaemagglutinin
RIA	Radioimmune assay
RSV	Respiratory syncytial virus
SCBU	Special care baby unit
SDS	Standard deviation score
SFGA	Small for gestational age
SPSS	Statistical programme for social sciences

INTRODUCTION.

Human cytomegalovirus (CMV) infection is world wide and present in all communities studied. This virus appears to have developed a symbiotic relationship with man and in the majority of individuals infection causes no disturbance to the health of the host. In circumstances where the host defence mechanisms are suppressed or immature this organism becomes pathogenic. This occurs in patients undergoing transplant surgery and in premature neonates. In this thesis the effect of intrauterine CMV on the fetus is explored together with the consequences of congenital CMV infection on affected children.

1. HISTORICAL BACKGROUND

At the beginning of this century intranuclear inclusion bodies were seen, at post mortem examination, in salivary glands of infants who died in the neonatal period. 92,117,155,169 It was felt that these infants may have had a protozoal infection and Smith 181 suggested the organism be named Entamoeba mortinatalium.

These lesions were again noted by Goodpasture and Talbot 66 in a six week infant, who died with bronchopneumonia; lesions were found in lung, kidney and liver. They coined the name "Cytomegalia" and suggested that the lesions were distinct and pathognomonic of a specific disease entity but questioned the protozoal origin. Viral etiology was suggested by Von Glahn 223 and proven, for the guinea pig form, by Cole and Kuttner 28, by transmission of the disease from material passed through a Berkefield N filter.

McCardock and Smith 123 and Smith and Vellios 183 presented a total of 46 autopsy reports of infants in whom intranuclear inclusions were seen, not only in the salivary glands, but also in other organs. Wyatt et al 235 reported a further six cases and reviewed all the previous case reports. They suggested viral etiology was likely in view of the similarity of the pathology to that found in mice infected with murine "Salivary gland virus". They also suggested that the inclusion bearing cells were pathognomonic

of the condition which they called "cytomegalic inclusion disease". Despite evidence from Farber 51 that these lesions were present in 12% of routine autopsies, Wyatt et al 235 considered that the condition was inevitably fatal. This theory was discounted by the observation of intranuclear inclusion bodies in epithelial cells in urine of non-fatal cases. 52,119,132

The possibility of investigating the true significance of CMV infection was realised, with the development of cell culture techniques, 50 and the isolation of murine CMV. 184 In 1956 human CMV was isolated simultaneously in three separate centres.

172,185,226

2. CYTOMEGALOVIRUS

The Herpes group of viruses include Herpes Simplex virus, Varicella Zoster virus, Epstein Barr virus and CMV. The common characteristics of the herpes group are their icosahedral (20 sided) shape, containing DNA and the ability to cause intranuclear inclusion bodies. 230 All Herpes group viruses exhibit the potential to establish latent infections. The morphology of CMV has been described by Wright et al 234 and the specific characteristics described by Weller 228 and further defined by Matthews. 121

3. DIAGNOSIS OF CMV INFECTION

The most reliable method of diagnosis of CMV infection is by virus isolation in cell culture. This has replaced identification of inclusion bodies in cells in urine which is less reliable. 52 The technique of cell culture is fully described by Albrecht and Weller. 7 Briefly 0.2 mls of fresh urine are inoculated into culture medium, in tubes containing a monolayer of human fibroblast cell culture. The tubes are then incubated for up to four weeks and inspected at regular intervals for evidence of the typical appearances of cytopathic effect (CPE). The sheet of elongated fibroblast cells show foci of enlarged, rounded cells with intranuclear inclusions. These foci then coalesce. The foci may appear between three days and two weeks incubation. CMV has been isolated from most body fluids, including urine, saliva, tears, breast milk, faeces, semen, lymphocytes and cervical secretions.

30,31,32,86,101,112

Because of the time required for isolation by cell culture, attempts have been made to develop more rapid diagnostic techniques. Schmidt and Gallo 178 developed an immunofluorescent technique whereby labelled antibody is introduced into cell cultures early to fix to CMV infected cells and so detect presence of the virus before CPE appears. Other similar techniques have recently been developed. 64,114,162

Alternatively, samples with high viral titres have been examined by electromicroscopy and virus has been detected in 90% of samples positive to CMV by cell culture. 114,118 This technique is of value in infants, less than six months old, who excrete large quantities of virus, but is less useful in older infants where the sensitivity falls to 45%, as most infants over this age excrete virus in titres of $<10^4$ organisms/ml. 114

4. SEROLOGICAL TESTS OF CMV

The complement fixation (CF) test is the most widely used test to detect CMV antibodies. The presence of CMV antibody (seropositive) indicates previous exposure to CMV, and may also be used to detect primary CMV infection by the detection of a rise in antibody in two samples tested in pair (i.e. seroconversion.) The technique was first used by Rowe et al 172 and has been fully described by Lennette and Schmidt. 116 In brief, CMV antigen, prepared from CMV positive cell cultures usually of strain AD 169, 33 is incubated with patient serum containing antibody, binding complement in this reaction. The remaining, unbound, complement then acts as an indicator of the degree of complement fixation by lysing sensitive sheep red cells. Stern and Elek 207 have shown cross-reactivity with many strains of CMV and Starr et al 204 have demonstrated no cross-reactivity with other viruses, except occasionally with other herpes viruses. 228 While individual titres of antibody may vary considerably with time, 224 Starr et al 204 have demonstrated that the titres remained positive ($>1:4$) in 19 of 21 children studied (76/78 tests) over a three year period. The major criticism of the CF test is its lack of sensitivity. This has led to the development of other techniques for the measurement of CMV antibody 70,160.

Indirect immunofluorescence (IF), 81,192 enzyme-linked immunosorbent assay (ELISA) 61,62 and radioimmune assay (RIA) 97,100 have been developed. All three tests have an enhanced sensitivity when compared with the CF test 70 but for routine screening and population studies it is debatable whether any one test is more valuable. 20,61,70,100,192

The major advantage of the newer tests is their ability to measure CMV specific IgM antibody which generally does not fix complement except in specific circumstances. 21 This is important for the diagnosis of congenital infection since IgG antibodies freely cross the placenta; so the CF test in the neonatal period will measure not only infant but also maternal antibodies. 73,192 Many viral infections are associated with a rise in IgM antibody levels in the acute phase of the illness. 96 The detection of CMV specific IgM has also been used to detect recent primary CMV infection. 97 All three methods of detection of IgM antibody have been used in both situations and a new method of IgM capture (MACRIA) has recently been described. 213

The sensitivity and specificity of the IF test appears to vary depending on the study. Ahlfors 4 found that 91% of primary infections, identified by seroconversion, were associated with raised IgM by IF, whereas other studies have identified only 60-69% by this technique. 69,74,85 In addition, false positive reactions are frequent. 42,74 Macroglobulin has also been associated with reactivation of CMV, and can be demonstrated in low titre intermittently for years using this assay. 100,179

Evaluation of solid phase RIA has demonstrated that this method will identify 86% of infections within four months of the primary infection 74 and is not positive in recurrent infection except in the immunosuppressed patient. 98,164 The period of persistence of IgM antibody, detected by ELISA in primary infections, has not been defined. 222,243 Although no direct comparisons are available between solid phase RIA and MACRIA or ELISA the sensitivity of each test is thought to be similar.

Similar problems have been encountered in the diagnosis of congenital infection using the IF techniques, with a 50-70% sensitivity rate, compared with virus isolation, 69,131 rising to 96% in symptomatic infants. 82 It is also associated with a high

false positive rate. 196 Solid phase RIA, however, is 93% sensitive, identifying 100% of symptomatic infants and 87% of asymptomatics. 75 The higher titres in symptomatic infants has led to attempts to predict those infants with a poorer prognosis using the level of IgM titre. 75 The ELISA technique has also been successfully adapted for use in this situation. 170

5. GENETIC VARIABILITY OF CMV

Serological tests of CMV antibody do not distinguish between individual strains of virus. 207 This has meant that it is not possible to prove epidemiological associations between related cases particularly in nosocomial infections. DNA endonuclease typing however will allow distinction to be made between strains. The technique was first described by Huang et al. 91 They demonstrated many different strains of CMV and identical strains only in viruses isolated from related cases, such as mothers, and their congenitally infected infants. They have also demonstrated minor genetic differences in viruses grown in cell culture over long periods. This technique has been further modified to allow application to samples with much lower virus titres. 56,219

6. CELL MEDIATED IMMUNITY (CMI) TO CMV INFECTIONS

Cell mediated immunity to viral infections is complex. It probably acts as the main defence mechanism against viral infections in humans. In CMV infection the numbers of T lymphocytes are normal and the measures of CMI to other antigens such as Herpes Simplex virus or PTA are normal. Interest was aroused by the use of CMV specific lymphocyte proliferation response (LTR) by Gehrz 57 as a measure of the competence of the CMI to combat CMV. This measure is mediated by T memory cells and results in their transformation to produce T and B lymphocytes which react with CMV antigen. 24 Pollard 159 has demonstrated that 90% of subjects, seropositive to CMV, have a positive LTR to CMV antigen. These studies have demonstrated that the LTR is suppressed or absent in the first few years of life in infants with congenital or neonatally acquired CMV. 60,143,147,148,168

This suppression is transitory and LTR function is restored to normal by four years of age, which coincides with the subsidence of

viruria. 206 A similar depression of LTR occurs in the last trimester of pregnancy and persists for three months postpartum. 59 This is not just in women who have CMV positive infants. No defect has been demonstrated in lymphocyte migration inhibition test 53 and lymphotoxicity assay. 171 Although the total T cell count is not reduced, the ratio of specific type T cells is altered in congenital and acquired CMV with a decrease in T8 (Suppressor) cells in infants under one year and restoration of Helper/Suppressor ratio in infants over one year. 149 The effect of these changes is not clear. The changes are similar in children with symptomatic and asymptomatic forms of congenital CMV and in post-natally acquired infection. 148 The suppression of LTR in pregnancy may allow CMV to reactivate and cross the placenta but this does not always occur. 59 The tests of lymphocyte function investigated to date may not be evaluating the critical steps in the cell mediated immune system which determine the presence or absence of viral replication, when replication may occur, and whether infection crosses the placenta.

7. EPIDEMIOLOGY OF CMV INFECTIONS

The epidemiology of CMV infection in Man is extremely complex and despite extensive study still not fully understood. The development of the CF test for CMV antibody by Rowe et al 172 allowed the prevalence of CMV infection to be studied. Since antibody persists for many years, the detection of antibody is a measure of previous exposure and so the percentage with detectable CMV antibody (seropositive) in the population rises gradually with increasing age. Rowe et al also observed a general trend to higher seropositivity rates in women compared with men.

Since these early studies, the prevalence of CMV seropositivity has been investigated worldwide and CMV antibody has been found in all populations studied. 17,102 These studies, summarised by Krech et al 102 showed a prevalence of antibody in adults approaching 100% in countries such as India, Uganda, Ivory Coast, the Caribbean Islands and Fiji, whereas prevalence rates of 40-60% were reported in Britain, USA, France and Germany. Other affluent countries, however, also have high seropositivity rate notably Japan (93%), Italy (97%), Czechoslovakia(92%), Finland(85%)

and Sweden (73%). 4 It is not apparent why there are these wide variations between countries, particularly where the populations are similar, such as Britain and Finland. Wide variations also occur, not only between populations, but between different subpopulations. 201,208

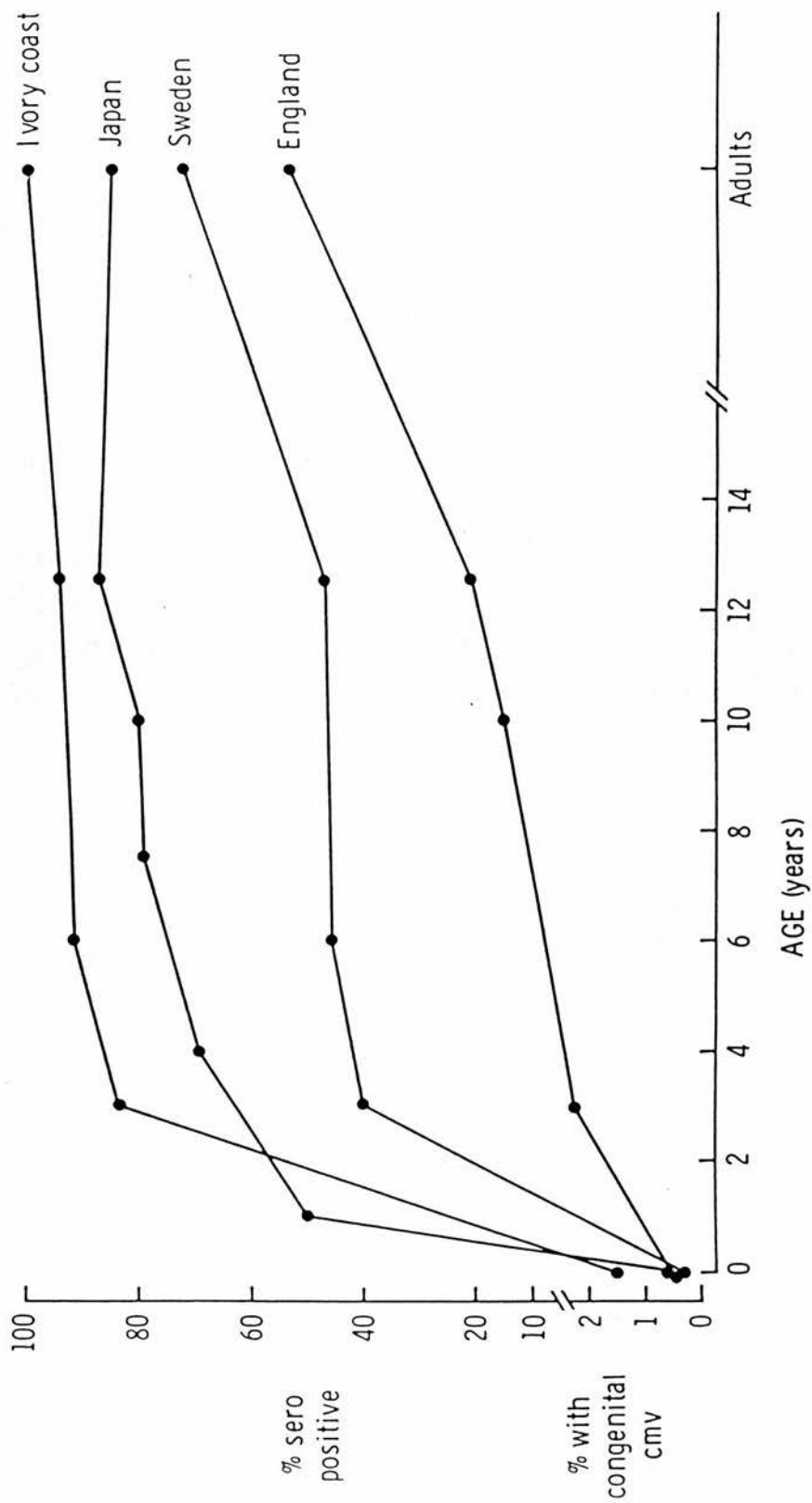
All the studies reported refer to pregnant women and there is a direct relation between the prevalence of antibody in pregnant women and the acquisition of antibody by children up to four years. 102 This is demonstrated in Fig.1, where it can be seen that infection is most rapidly acquired in Japan and Ivory Coast, where the prevalence in adults is also high, and least rapidly in Britain.

Acquisition in the first year of life

There is now substantial evidence that the major source of CMV in young children is their own mothers. In population studies Granstrom et al 68 identified 51 (32%) children who excreted CMV in a cohort of 148 children followed for one year. None of the infants born to the 23 seronegative mothers acquired CMV. Similar findings have been reported from France, 109 Japan 95 and Britain. 93 That the virus acquired is of maternal origin is proved by DNA endonuclease typing, which shows identical strains of virus isolated from mother and baby. 154 There are two major sources of infection; that acquired at birth by passage through an infected birth canal, and infection acquired from breast milk. In healthy women CMV is more frequently recovered from cervix than any other site. Isolation rates of up to 15% have been reported.

101,137,193 High isolation rates have been related to lower social class and young maternal age. 101,137 Montgomery et al 137 also reported isolation rates increased in pregnancy towards the third trimester but Stagno et al, 193 using nonpregnant matched controls, demonstrated that the isolation rates were suppressed in early pregnancy and actually increased to prepregnancy levels in the last trimester.

Infants who acquire CMV during delivery do not start to excrete virus until after an incubation period of at least 21 days. 166 Thus congenital and acquired infection can be distinguished. In a study by Stagno et al, 193 acquisition of virus was more



Prevalence of congenital CMV infection and CMV antibody by age in different countries.
FIG 1.

frequent in infants of mothers who excreted virus from the cervix in the last trimester and post term (57%) compared with those who excreted virus in trimesters I and II (12.5%). Infants of women who excreted virus in urine and saliva only, but not from the cervix, did not acquire CMV in this study 193.

Breast milk is the other common source of infection. Hayes et al 86 isolated CMV in 27% of samples of breast milk from seropositive women. The frequency of isolation was higher in breast milk after one week than in colostrum samples. 197 In a study of the infants of 396 mothers, 197 57% acquired CMV when breastfed by mothers whose breast milk was known to contain virus. None of the nine bottle-fed infants acquired the infection, despite active maternal infection. The rate of acquisition was most rapid in the first six months of life and declines after this age.

68,109,142

CMV may also be acquired in the neonatal period from sources other than the mother. Nosocomially acquired infection occurs, though it has not been possible to document cross-infection in neonatal nurseries. One incident investigated by Gurevich et al 79 was heavily criticised as no DNA typing was performed, and other, more likely, sources of infection were not excluded. 189 Spector, 190 however, has recently investigated seven cases of CMV infection in a neonatal unit using DNA endonuclease typing. Three babies were infected with an identical strain from either the same source or spread within the nursery. Cross-infection was not universal as four other babies, with temporally related acquisition of CMV, were found to be infected with four different strains.

Blood transfusion is a more frequent source of acquired CMV infection in neonatal units. 237 This was confirmed in two studies 14,145 where CMV was acquired by 16-35% of infants given blood with CMV antibodies but less than 7% acquired CMV if CMV positive blood was not used. The remaining infections were presumably maternally acquired. Neonatal infection is usually symptomless and of no clinical significance, but when acquired by ill or very low birth weight infants CMV can result in acute illness with lymphocytosis, hepatosplenomegaly, respiratory distress and death. 12,236

The rate of CMV infection was similar in infants of seropositive mothers irrespective of blood transfusion. 240 Of 74 seronegative infants, however, 13.5% receiving seropositive blood acquired CMV but none of those receiving seronegative blood acquired infection. The rate was also related to the volume of blood given. Acquisition of CMV by this route is more serious for infants of seronegative mothers because of the lack of CMV antibodies acquired by passive transfer. Even infants, who are under 1500g, of seropositive mothers, are at risk of serious sequelae from CMV because of the rapid fall in titre of passively acquired CMV antibody due to catabolism and blood sampling. 242

Acquisition in older children.

Acquisition of CMV in older children may be from sources outside the family. Since infants with both congenital and postnatally acquired CMV excrete large quantities of virus for periods of up to four years, 192 siblings or play contacts provide multiple sources of infection once children become mobile and start to mix with other children outside the home. Children who attend day nurseries acquire CMV infection more rapidly than children cared for full time in the home. 150 In studies from Alabama, 151 in a white, middle class, population, only 10% of infants under one year were excreting CMV, but this rose to 78% between one and two years in those cared for full time in a day nursery compared with only 18% of similar children cared for at home. Further evidence comes from a study of Israeli children from three different communities. 176 Using serology to assess the frequency of infection, there was a steep rise in seropositivity in urban children when they first attended day nurseries, and this was exacerbated by overcrowding. In bedouin children, however, who live in isolated seminomadic communities until they start school, the prevalence of antibody rises steeply at five years. Children in a kibbutz, however, were subjected to a communal care system from about six months and, in consequence, acquired CMV much earlier than either urban or bedouin children. The prevalence of antibody gradually rises in later childhood and is probably influenced by exposure to siblings and class mates leading to higher rates of acquisition in institutions. 207

In contrast with congenitally infected children, post natally acquired CMV is not associated with serious neurological handicap, although transient illness such as hepatosplenomegaly and pneumonitis may occur. 80,108

Acquisition in young adults

Circumstantial evidence suggests that sexual transmission is a common mode of transmission in young adults. This is based on the rapid rise of seropositivity in the teenage years. 39 CMV is frequently found in association with patients attending sexually transmitted diseases clinics, 94 and very high rates of antibody are found in homosexual men. 45 CMV mononucleosis has been reported following sexual contact 27,110 and CMV has been cultured from semen 112 while higher rates of isolation from the cervix are reported in teenage girls with rates decreasing with age. 101 Finally the rise in antibody levels, seen in young adults, does not occur in nuns working as nurses or teachers. 39

Epidemiology of CMV infection in pregnancy and its relationship to rates of congenital CMV.

The prevalence of congenital CMV varies from 0.24% to over 2% of live births depending on the population screened (Table 1). There is a general trend for high rates of congenital infection to be associated with high seropositivity rates in the adult population. In England, 127 Denmark, 9 and Canada, 113 where rates of congenital infection of 0.2-0.4% of live births are reported, the prevalence of antibody in the adult population is low. In developing countries such as Chile 202 and the Ivory Coast 180 and disadvantaged subpopulations in the USA, 201 where adult seropositivity rates range from 82-100%, rates of congenital infection of 1.4-1.7% of all live births are reported. This suggests that poor social circumstances lead to an increase in both congenital CMV and adult CMV infection. However, this is not always the case. High rates of both congenital CMV and adult seropositivity are seen in economically affluent Finland, 68 whereas in Japan and Sweden there is a low rate of congenital infection despite very high seropositivity in mothers. 5,95 Thus the relationship between the prevalence of congenital infection and the

TABLE I
Rates of congenital CMV and prevalence of maternal CMV antibody

	Rate of congenital CMV(%).	Percentage of mothers seropositive.
Manchester <u>127</u>	0.24	25
Denmark <u>9</u>	0.4	53-65
Sweden <u>5</u>	0.4	71-79
Canada <u>113</u>	0.4	44
Japan <u>95</u>	0.5	94
London <u>209</u>	0.5	56-64 (seronegative mothers only)
U.S.A. <u>201</u>	0.6	55 ("middle class")
Ivory Coast <u>180</u>	1.4	100
U.S.A. <u>201</u>	1.6	82 ("lower class")
Chile <u>202</u>	1.7	98 (small sample)
U.S.A. <u>203</u>	1.7	65 (mothers <20yrs.)
Finland <u>68</u>	2.0	85 (small sample)
U.S.A. <u>194</u>	2.2	82 (seropositive mothers only)

prevalence of maternal seropositivity is more complex than it at first appears.

The rate of primary infection in pregnancy varies and is reflected by the rise in the prevalence of antibody during the childbearing years. 69,71,208 Rates of seroconversion during pregnancy vary from 0.6-4.0%. The average rate of seroconversion in pregnancy in serosusceptibles in published studies is 1.1%. 200 In communities with high seropositivity rates, the chance of seroconversion for the remaining serosusceptibles may be high. Fetal infection occurs in about half of these cases studied, though it is not yet possible to predict which infections will be transmitted in utero (table 2).

In the early studies it was assumed that previous maternal CMV protected the fetus from congenital CMV. 208 The finding that congenital CMV could occur in consecutive siblings demonstrated that reactivation of latent infection or reinfection could result in intrauterine infection. 49 Huang et al 91 demonstrated, using DNA endonuclease typing, that the infection in a pair of congenitally infected siblings was due to infection with genetically identical virus in both infants. This suggested that infection in the second pregnancy resulted from reactivation of latent virus, not reinfection with a new strain. This type of infection is now referred to as a recurrent maternal infection. In communities with high rates of congenital CMV the majority of cases of congenital CMV result from recurrent maternal infection. 95,180,202 This has been confirmed by Stagno et al 194 who report a rate of congenital CMV of 1.9% in a study of women known to be seropositive prior to pregnancy. The overall rate of congenital CMV in the community was 2.2%

The overall rate of congenital CMV therefore depends on the rate of primary maternal infection as well as the rate of recurrent infection during pregnancy. The distinction between the relative contribution of each type of maternal infection to the rate of congenital infection is difficult. It is rarely possible to obtain preconceptional blood specimens to exclude an early primary infection in women already possessing antibodies to CMV when presenting for antenatal care. Grant et al 69 and Stern and Tucker 208 found no congenital infections following recurrent

TABLE 2.
Frequency of intrauterine transmission of primary maternal CMV infections.

	n.seronegative women screened.	n.primary CMV infections (%)	n.congenital infections (%)
Monif <u>136</u>	604	4(0.6)	4(100)
Stern <u>209</u>	270	11(4.0)	5 (45)
Grant <u>69</u>	1841	13(0.7)	5 (38)
Kumar <u>106</u>	1404	14(1.0)	7 (50)
Giffiths <u>76</u>	1608	14(0.9)	3/12(25)
Ahlfors <u>5</u>	1218	14(1.2)	6 (43)
Total	6945	70(1.0)	30 (44)

infection, as judged by the presence of viruria in women who already possessed CMV CF antibodies. Stagno et al, 201 however, reported that among a high income group, with a seropositivity rate of 55%, 53% of congenital infections followed primary maternal infection and the remainder (47%) followed maternal recurrent infection. In contrast, in the low income group, with a seropositivity rate of 82%, only 19% of congenital infections followed maternal primary infection and 81% following maternal recurrent infection. In this study, the overall rate of congenital CMV in the middle income group was 0.6% and, in the lower income group, 1.6%.

This high rate of congenital CMV does not occur in all highly seropositive populations. In Japan, 95 where all congenital infections studied resulted from maternal recurrent infection, the overall rate of congenital CMV is 0.5%, three times less than that reported in the Alabama study. 201 The different rates of recurrent maternal infection, resulting in congenital infection, may in part be accounted for by different age and socioeconomic structures of the pregnant population. Recurrent infection is more prevalent in young women, black women, and may also be related to social class. 101,137 All these are features of the lower socioeconomic class group studied by Stagno et al 201 and this could account for the high rates in their study. The lower rates in Japan could result from differences in maternal age and socioeconomic status but, unfortunately, the population screened was not described in detail. In Sweden, 5 with a similarly high prevalence of antibody and low rates of congenital infection, as in Japan, the proportion of congenitally infected infants whose infections could be attributed to recurrent maternal infection was only 36%.

The rate of congenital infection, within a particular community, therefore depends on the rate of primary infection in the childbearing years and the overall seropositivity rate. The age of mothers, their race, socioeconomic status and perhaps genetic factors, influencing host immune control, will then influence the frequency of primary infection, recurrent infection coinciding with pregnancy and the rate of intrauterine transmission.

8. PATHOLOGY OF CMV INFECTION

The specific characteristic of CMV is its ability to cause cytomegalia in infected cells. 89 The affected cells are characteristically of epithelial origin and are 2-4 times the size of surrounding cells. They have an eccentrically placed nucleus which is large (10 -15µm) and contain an intranuclear inclusion (8-10µm) presenting an "Owl's eye" appearance. These giant cells may be associated with cellular infiltrate of plasma cells and lymphocytes. 89 Intranuclear inclusions of CMV have been seen in many organs, in biopsy and post-mortem specimens, from children dying of cytomegalic inclusion disease 183 and in children who die from other conditions who are coincidentally infected with CMV. 51

Intranuclear inclusions are most commonly seen in the salivary gland where cytomegaly is confined to the ductal epithelium. Cytomegalic cells are found in 12% of routine autopsies 51 but appear to be less common in children over 10 years. 216 In the gastrointestinal tract, giant cells may be seen but may be obscured by the presence of enterocolitis. 183 In the liver of newborn infants, giant cells are seen in the bile duct epithelium. There is also extra medullary haemopoiesis and infiltration of the portal tracts. The liver parenchyma is not involved. Acquired CMV, giving rise to hepatitis, and mononucleosis, is associated with mononuclear infiltration without giant cell formation and good preservation of liver architecture.

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In the lungs, giant cells are seen in alveolar and bronchial epithelium. This is one of the most common sites of symptomatic acquired CMV 188 particularly in infants and the immunosuppressed. Renal involvement is seen in the interstitium with clusters of giant cells in the proximal tubules. This is likely to account for the universal association of viruria with all forms of CMV disease. Petechial and purpuric skin rashes are sometimes seen in congenital infection, but vesicular lesions similar to herpes simplex have been reported. 18 Maculo-papular rashes may also complicate CMV mononucleosis. 99

Graham et al 67 noted bone changes in an infant with congenital CMV, similar to congenital rubella, with linear lucent

areas between zones of sclerosis in the distal metaphyses ("Celery stalking"). Spontaneous fractures have also been noted in severely affected neonates. 187 A specific lesion of teeth has also been described by Stagno et al 203 with opaque enamel, rapid wear and severe caries.

Central Nervous System Involvement

Severe congenital CMV is typically associated with CNS involvement causing microcephaly or hydrocephaly, periventricular calcification, focal softening, haemorrhages and subependymal inclusion bodies. 88,183,235 Microgyria due to small abnormal gyri have also been described. 34 This type of malformation must develop before the sixth month of intrauterine life. Neurological involvement is not always obvious in neonates who subsequently develop neurological handicaps. Bray et al 22 has suggested that the neurological involvement may be progressive, and describe two children who had normal or minor changes only on computerised tomography (CT) which progressed to severe extensive disease over three months. Although CT scan may demonstrate abnormalities not apparent on physical examination and so suggest a poor prognosis, 8 it would be rash to claim absence of CNS involvement on the evidence of a normal CT scan alone.

Congenital CMV has been associated with several types of visual defect ranging from anophthalmia, 124 microphthalmia, to optic atrophy. 55 These lesions may be associated with peripheral choreoretinitis, or occur alone. Choreoretinitis has not been described in isolation in an otherwise asymptomatic child with CMV, in contradistinction to toxoplasmosis. 195 Peter's anomaly (i.e. a central corneal defect associated with adhesion of the lens) has also been reported. 55

Congenital CMV is a major cause of deafness. 195 This is due to a viral labyrinthitis affecting the vestibular and semi-circular canals and the epithelial cells of inner ear, resulting in hydrops of the inner ear, 138 but not the organ of corti. 41 Electronmicroscopy has shown this to be associated with virus particles 37 and is distinguishable from rubella labyrinthitis. 210 CMV has been cultured from endolymph. 40 Animal models suggest that virus may invade the inner ear via the eighth cranial nerve 38 rather than via blood, as is the suggested

route for rubella virus. Progressive damage may also occur as a result of congenital CMV infection, as with rubella. 36

9. SPECTRUM OF DISEASE IN CONGENITAL CMV

The majority of children with congenital CMV are asymptomatic at birth. Initial studies described the natural history of children presenting in the neonatal period with symptoms of congenital infection i.e. cytomegalic inclusion disease (CID). It is now recognised, from prospective studies, that some children with asymptomatic disease at birth may develop significant disease in later life

a) Cytomegalic inclusion disease.

Congenital CMV was first suspected in infants with abnormalities noted at birth, and the majority were only recognised at autopsy. 123,235 These findings have been fully described by Medearis. 130 Following the realisation that infants with severe congenital CMV infection could survive, 119,132 several authors described series of patients with cytomegalic inclusion disease. 125,146,232 McCracken et al 125 reported on 20 children with CID. Although two may have acquired infection from blood transfusion, he was able to comment on the long-term follow-up of 18 children with confirmed CID. Sixteen of 18 (89%) presented with hepatosplenomegaly, 13 of 14 (93%) thrombocytopaenia and 61% (11 of 18) haemolytic anaemia. Nine (50%) presented with evidence of CNS involvement.

Pass et al. 146 reported the long-term follow-up of 34 infants with symptomatic congenital CMV, 25 (73%) presented with hepatosplenomegaly and 19 (56%) with evidence of CNS disease. Both Pass et al and McCracken et al showed similar results at long-term follow-up. Mortality was 22% 125 and 29% 146 in each series. Most deaths occurred in the first year of life and all occurred in infants with severe CNS disease such as cerebral palsy and microcephaly. In all but one, evidence of extra CNS disease had resolved by the time of death. The exception was noted to have hepatic cirrhosis. 146 Hepatosplenomegaly may persist for up to four years with a wide range of pathological processes such as cholangitis and periportal fibrosis. 125 Despite the usually benign course of CMV hepatitis, one other fatal case of portal

fibrosis, with oesophageal varices, has been reported. 44 Other extra CNS manifestations usually resolve spontaneously though thrombocytopaenia may persist for more than three months. 125 CMV pneumonitis is usually benign. 125 There is one case report of a 13 month-old child who developed diabetes mellitus associated with congenital CMV 225 and one of sudden death at five weeks due to myocarditis in an infant with CID. 212 Extra CNS disease may not be present at birth, a prospective study by Starr et al 205 noted no children with hepatosplenomegaly at birth but one child had splenomegaly at six weeks.

A number of studies have suggested an association with congenital abnormalities. Occasional congenital heart defects have been noted 15,122,125 and other sporadic malformations such as imperforate anus, 205 cleft palate and micrognathia reported 125 but no recognisable pattern of congenital defects.

The long-term neurological outcome of symptomatic infants is very poor. In all three long-term studies 125,146,232 the rate of major neurological handicaps was very high. Fourteen (60%) of 23 survivors in the Alabama study suffered from spasticity and fits, seven had normal IQ but deafness, and only two were normal (6% of total, 9% of survivors.) McCracken et al 125 reported only four (22%) to be normal at nine years, four suffered from bilateral sensorineural deafness, and seven (39%) severe psychomotor delay with spasticity and/or fits.

Williamson et al. 232 followed 17 infants with CID, nine (53%) suffered from severe CNS disease, a further six, with normal IQ, deafness, and only two (12%) were normal at four years. The overall rate of deafness was 65% and choreoretinitis 41%. In addition three suffered from specific language disorders and four had learning difficulties despite normal intelligence.

b)Prospective studies of congenital CMV

Table 3 shows the major prospective studies of the prevalence of congenital CMV. As noted in section 6, there is a wide variation in the rate of congenital infection depending on the population studied. The possible reasons for this have already been discussed. The method of screening may also affect the rate of detection of cases. Several populations have been screened several times. Melish and Hanshaw 131 found a tenfold increase

TABLE 3
Prospective studies of congenital CMV infection.

	Diagnostic method.	n.Mothers screened	n.Congenital CMV(%)	n.Symptomatic at birth(%)
Denmark <u>9</u>	V.I.	3060	10(0.33)	0
New York <u>131</u>	V.I.	1963	20(1.0)	2(10)
	CMV IgM	1845	2(0.1)	1(50)
New York <u>83</u>	CMV IgM	8644	53(0.6)	0*
Sweden <u>1</u>	V.I.	2200	7(0.3)	2(28)
Hamilton <u>113</u>	V.I.	15212	64(0.4)	4(6)
Cleveland <u>206</u>	V.I.	2259	26(1.6)	1(4)
Alhabama <u>167</u>	Total IgM	9100	22(0.24)	0*
Alhabama <u>201</u>	V.I.	2698	16(0.6)	- 1.
	V.I.	1014	16(1.5)	- 2.

V.I.- virus isolation

* - symptomatic infants excluded

1. - "middle class"

2. - "lower class"

in prevalence using virus isolation compared with CMV specific IgM measurement, using IF, on cord blood samples. This method is more likely to select seriously affected infants 75 and, indeed, in this study 50% of infants identified by CMV specific IgM were symptomatic at birth compared with 10% diagnosed by virus isolation. 131 Hanshaw et al. 84 re-studied this population with an improved CMV specific IgM IF test but the rate of congenital infection was still significantly less than previously noted by virus isolation.

Similarly, studies in Alabama 167 used increased total IgM as the screening method. These infants were then re-tested specifically for CMV by virus isolation. The rate of congenital infection (0.24) was much lower than that found in the same population by Stagno et al. 201 The sensitivity of the screening test will therefore have an effect on the findings of each study and the rates of handicap identified as a result of congenital infection.

c)Long-term Sequelae of Congenital CMV

(i)Deafness

The incidence of deafness in children with congenital CMV who present with symptoms in the neonatal period ranges from 11-65%, 6,125,195,199 with a mean of 28% (Table 4.) All suffer from moderate to profound deafness and 70% are bilateral. The highest rate was reported by Williams et al at 65%, 232 and the lowest in the Collaborative Study at 11%. 127 These major differences are likely to be due to the criteria used to define symptomatic infection.

Children with asymptomatic congenital CMV at birth have a much lower incidence of deafness (Table 5.) The range reported is wide (14-50%.) The rates are high in some studies where children with very minor hearing losses are included. In the study by Dahle et al, the inclusion of three infants who suffered loss at 8KHz only resulted in an overall rate of 50%. 35 When only those with significant deafness, i.e. >50 dB bilateral loss and likely to require hearing aids or special schooling are included, the rate falls to 5.5%. The range for all studies, of significant deafness, is 0-7.8% (Table 5).

TABLE 4
Prevalence of Deafness in Children with Congenital CMV,
Symptomatic at Birth.

	n.CMV	Deaths	Deafness in survivors		
			1x	2x	Total(%)
McCracken <u>125</u>	18	4	-	4	4(29)
Pass <u>146</u>	34	10	3	4	7(29)
Stagno <u>199</u>	24	-	4	4	8(33)
Williamson <u>233</u>	17	-	1	10	11(65)
McDonald <u>127</u>	49	4	-	5	5(11)
Ahlfors <u>6</u>	15	1	-	3	3(21)
Total	157	19	8	30	38(27)

Note 1x :Unilateral deafness
2x :Bilateral deafness

TABLE 5.
Prevalence of deafness in children with congenital CMV,
asymptomatic at birth.

	n.CMV	n.Deafness (%)	Mild(25-55db)		Moderate-profound (>55db)	
			1x	2x	1x	2x(%)
Dahle <u>35</u>	18	9(50)	3	3	2	1(5.5)
Stagno <u>195</u>	51	7(14)	-	1	2	4(7.8)
Hanshaw <u>83</u>	40	5(12.5)	-	2	-	3(7.5)
Saigal <u>175</u>	40	6(15)	1	1	2	2(5.0)
Kumar <u>107</u>	17	4(23)	2	1	1	-(0.0)
Total	166	31(19)	6	8	7	10(6.0)

Note 1x:Unilateral deafness
2x:Bilateral deafness

There is some evidence that sensorineural deafness, associated with congenital CMV, may be progressive. Williamson et al 232 reported three children with mild sensorineural loss, at less than two years, which progressed to severe bilateral deafness at three years. Similar findings have been reported by Dahle et al. 36

(ii) Psychomotor Development and Intelligence

The evidence that congenital CMV infection is a cause of mental handicap is conflicting (Table 6.) Melish and Hanshaw 131 suggested that congenital CMV caused as much mental handicap as Down's syndrome. This was based on their finding that two infants identified from a population of 1845 had severe psychomotor delay, a prevalence of handicap 1/1000 equivalent to the rate of Down's syndrome in Britain. Congenital CMV may cause severe neurological sequelae 125,130,146,232 but it is not clear if there is a specific effect on intelligence in the absence of other neurological problems. Four prospective studies have examined the intellectual ability of children with congenital CMV.

83,107,167,175 In two studies 103,107,175 the IQ tests were repeated on two occasions and there was no difference in the intellectual abilities of children with congenital CMV and their matched controls. Both studies had difficulty in matching and there was a higher proportion of single mothers of poor educational background amongst the congenitally infected infants than amongst the controls. This would have made any differences between the groups even more apparent if CMV had an effect. Both studies also included a battery of other psychometric, language and behavioural tests, and neither showed any significant effect of congenital CMV apart from an increase in conduct disorders in the Canadian study. 175 This was probably related to the matching problems previously referred to.

Reynolds et al, 167 while not showing a significant difference in the IQ between congenitally infected infants and controls, postulated that the results were suggestive of an adverse effect of CMV, because the mean IQ of congenitally infected infants was lower than controls. Indeed, this study included one congenitally infected child with severe spastic quadriplegia. The only study to demonstrate a significant effect of CMV on intellectual development is that reported by Hanshaw et al. 83

TABLE 6.
Congenital CMV: Effect on intelligence.

	Test used	n.CMV	mean age yrs.	I.Q.CMV	I.Q.Controls
Kumar <u>103</u>	Stanford Binet	13	4.0	85.0	86.5
Kumar <u>107</u>	WISC/ WIPPSI	17	7.6	89.5	85.9
Reynolds <u>167</u>	Stanford Binet	18	3.2	92.0	100.0
Hanshaw <u>83</u>	WISC/ WIPPSI	44	3.5) 7.0)	105.0	114.0* 113.0*
Saigal <u>175</u>	Stanford Binet	47	3.0 5.0	97.0 107.0	100.6 106.5

* Matched controls = 114.0 p<0.025 compared with CMV
Random controls = 113.0

One child with severe cerebral palsy was included in the testing, which makes it difficult to assess the effect of congenital infection on intellect alone. Hanshaw predicted "school failure" on the basis of IQ <90, or abnormal neurological findings including deafness. 36% of children with CMV were expected to fail at school compared with 14% of matched controls. However, all those who were likely to fail were cases and controls from social classes IV and V suggesting an environmental effect, particularly on those with an IQ of 80-90.

(iii) Specific Neurological Features

The poor neurological outcome of infants, symptomatic at birth, has already been discussed. Each study reporting the outcome of infants who are asymptomatic at birth have described individual children with microcephaly, spasticity, and fits with rates varying from 2 - 6.6%. 6,83,103,167,175 More detailed examination of three symptomatic infants by Williamson et al 232 suggests that they may have suffered from specific speech defects such as dyspraxia or dysarthria. These authors have suggested focal damage may have occurred in the motor speech area. Visual disorders such as optic atrophy and choreoretinitis have been described, but these are rare and occur in children with other handicaps due to congenital CMV. 55,124,195 In addition to deafness, minor vestibular abnormalities have been demonstrated in otherwise asymptomatic children with congenital CMV.

d)The Relationship of Primary and Recurrent Maternal CMV on Outcome of Congenital Infection.

In the six prospective studies of primary CMV infection in pregnancy, 5,69,76,106,136,208 the rate of intrauterine transmission is about 50%, with two exceptions (Table 7.) In the study by Monif et al, all of four mothers with primary CMV infection transmitted in utero. 136 Griffiths and Babbonia 76 however reported a very low rate of transmission (17%.) This study, however, consisted of two groups of pregnant women, those who were seronegative at first antenatal attendance who experienced seroconversion, and 2486 unselected women, seropositive at booking, who were re-tested for CMV specific IgM. Thus early infections occurring up to 16 weeks before the first antenatal attendance were

TABLE 7.
Maternal CMV infection and relation to subsequent outcome
in children with congenital CMV.

	n. Maternal primary infections	n. Congenital CMV	<u>Trimester</u>			Children with symptomatic infections (trimester)
			I	II	III	
Monif <u>134</u>	4	4	0	2	2	2(II)
Stern <u>209</u>	11	5	2	2	1	1(II)
Grant <u>69</u>	13	5	3	2		0
Kumar <u>106</u>	14	7	0	2	5	1(III)
Griffiths <u>76</u>	45	8	2	0	6	1(III)

diagnosed. In this group the rate of intrauterine transmission was significantly lower than those experiencing a seroconversion.

161 This study suggests that first and second trimester infections may cross the placenta less frequently than late infections. 133

Although symptomatic congenital CMV may follow primary infection in all three trimesters 76,106 it has been suggested that early infections are more severe. 5,105,136 Alhfors et al. 6 reviewed 46 cases of congenital CMV, nineteen following maternal primary infection. First trimester maternal infection resulted in symptomatic congenital CMV in all five children and all have subsequent neurological handicap. However, only one of these five cases was diagnosed in a prospective study, and the remainder were referred from other centres.

Stagno et al. 201 reported symptoms at birth in five of 33 infants with congenital CMV following maternal primary infection. This was from a mixed population, some screened prospectively, and some referred. The symptomatic infants, however, followed infections occurring in all three trimesters.

Recurrent maternal infection was originally thought not to give rise to symptomatic congenital infection. There have been four case reports however, where infants have been severely handicapped following a confirmed maternal reactivation.

3,87,173,239 In prospective studies it is difficult to classify the type of maternal infection, as pre-pregnancy blood samples are rarely available. The presence of CMV specific IgM in the first antenatal blood sample suggests early primary infection, but the absence of CMV specific IgM, particularly by some assay methods, does not exclude primary infection (see section 3.) In the prospective study by Alhfors 5 42% of maternal infections could not be classified in either primary or recurrent groups. The use of more sensitive and specific methods to detect CMV specific IgM such as the RIA test has allowed more accurate definition. Stagno et al 201 found no symptomatic infants in the 27 congenitally infected infants following maternal reactivation compared with five of 33 following maternal primary infection. Thus maternal reactivation during pregnancy appears to carry a lower risk of serious handicap in congenital CMV but the number so far followed

up in prospective studies remains small.

10. PREVENTION AND TREATMENT OF CYTOMEGALOVIRUS INFECTIONS

CMV is usually a benign infection in healthy children and adults. Prevention or treatment of infections is important only for those groups who are susceptible to symptomatic infection, such as low birth-weight neonates, immuno-suppressed individuals, and infants in utero. The acquisition of CMV from blood transfusion has been prevented by restricting blood products given to seronegative subjects, to products from CMV seronegative donors. This has nearly eliminated severe acquired CMV infections in neonatal intensive care units. 14,104,240 The matching of renal transplant recipients by CMV status, with the donor organ, has reduced, though not eliminated, CMV as a serious opportunist organism in the post-operative immuno-suppressed period. 16

The multiple sources of CMV to which a pregnant woman may be exposed makes it difficult to prevent the acquisition of primary CMV in pregnancy. Indeed, there is little evidence that professionals exposed to known sources are at greater risk than the population as a whole 2,47 and attempts to reduce exposure of nurses to known cases of CMV has been found to be impractical and unnecessary. 244

Passive Immunisation

Immunoglobulin has been used in the immuno-suppressed to alter the natural history of CMV infection. In a study of bone marrow transplant patients, Winston et al 233 were not able to prevent the acquisition of CMV by the administration of 5% immunoglobulin solution. Symptomatic infections and pneumonia appeared to be reduced in the treated group. High titre CMV specific immunoglobulin may, however, have a greater role to play. Condie et al 29 completely prevented the acquisition of CMV by bone marrow transplant recipients with repeated administration of high titre specific immunoglobulin in the first 120 days after transplantation. They found no protection if non-specific immunoglobulin was given. This benefit could not be maintained, however, if granulocyte transfusion were given from CMV positive donors, demonstrating that immunoglobulin has no effect if the organism is protected by donor cells during acquisition. 129

High titre CMV specific globulin has also been used to modify symptomatic CMV infections in renal transplant patients 141 and, in one case report, presumed blood transfusion acquired infection in a premature infant. 186 The scarcity of CMV specific immunoglobulin makes it unlikely that this form of treatment has any role in prevention of congenital infection.

Vaccination

A successful live attenuated vaccine has been developed for murine CMV 135 The first attempts to develop a vaccine for human CMV was by Elek and Stern. 48 They used strain AD 169 and subjected it to 56 passages to attenuate the virulent virus. The "vaccine virus" was then administered to 26 volunteers and 25 subsequently had a rise in CMV antibodies. The antibody produced showed cross-reactivity to not only AD 169 strain, but to several other wild and laboratory strains. No live virus was isolated from urine or throat swab, suggesting that the vaccine virus had not established latency. 140

Plotkin et al 156 similarly subjected the Towne strain to 129 passages to produce a vaccine. This vaccine virus strain stimulated humeral immunity when given subcutaneously but not intranasally. CMV specific cell mediated immunity was also transiently stimulated. This vaccine has now been extensively tested in renal transplant subjects. 63,158 It has not been shown to prevent the acquisition of CMV but it may modify the severity of complications such as pneumonitis. The vaccine strain, however, has been shown to be safe and not to cause latent excretion itself despite immunosuppression in all the subjects. The vaccine has not been tested on pregnant subjects, though it has been given to paediatric nurses. 54 This resulted in a similar antigenic response but the numbers were too small to demonstrate the efficacy of preventing the acquisition of wild infection and there is no data on the availability of the vaccine to prevent congenital infection.

Antiviral Drugs

Cytosine arabinoside has been used in children with symptomatic congenital CMV infection. 126 In large doses, viral excretion was temporarily suppressed, but there was no symptomatic change in the infants. All had, by definition, severe disease,

and doses which suppressed virus excretion were too toxic for long-term use.

Adenine arabinoside, which is used in the treatment of severe herpes simplex infections, is not effective against human CMV. It has a similarly suppressive effect on CMV excretion but little clinical benefit 26 although occasional cases have improved. 35 Severe toxicity has been noted in renal transplant patients. 120

Acyclovir, which is highly effective against herpes simplex virus, is much less active against CMV. 218 The drug requires intracellular activation with the enzyme thymidine kinase. Most herpes group viruses stimulate the production of this enzyme in infected cells, but not human CMV. 177 The drug is effective in preventing death from murine CMV in immunodeficient mice 23 but it has a much less marked effect in human infections. Plotkin et al 157 have demonstrated that in large concentrations the drug inhibits CMV replication by 90% in vitro but had great difficulty in achieving similar serum concentrations in four infants with congenital CMV infection. Doses of 15 mg/kg produced temporary reduction in viruria but no clinical effect. Yeagar et al 241 only achieved "therapeutic" levels in one of four infants with CMV. This child improved but had also been given CMV specific immunoglobulin. The other three infants showed no clinical improvement. Acyclovir may therefore eventually prove to be a useful drug if high doses can be used, and new analogues may be more effective in the future. 220

PATIENTS AND METHODS.

Cytomegalovirus infection is the commonest known congenital infection. The prevalence of maternal infection and the incidence of congenital CMV vary widely throughout the world. While the majority of congenitally infected infants are asymptomatic at birth, a proportion develop significant handicaps in later life. As yet, there is no method of prevention or treatment of infection available to pregnant women or their infants, and no agreed policy on management of CMV in pregnancy. It was, therefore, necessary to conduct a prospective study to assess the risk of acquisition of CMV in pregnancy, the rate of intrauterine infection and the prevalence of congenital CMV on a British population, to assess the extent of the morbidity resulting from CMV in pregnancy. The aim ultimately was to establish whether congenital CMV infection was a sufficiently serious public health problem to justify attempts at prevention by vaccination. This thesis reports the findings of the follow-up of children with congenital CMV, up to three years of age, identified between 1.3.80 and 30.6.83, and assesses the impact of congenital CMV on this population.

1. POPULATION SCREENED

(i) As part of a study of the epidemiology of CMV infection in pregnancy, all women attending three maternity units in London were screened for evidence of previous CMV infection. At their first antenatal visit, a questionnaire was completed. Details of age, parity, marital status, race, country of birth, maternal and partner's occupation were obtained at interview by the midwife (Appendix 1). A blood sample collected at this visit was tested by complement fixation (CF) test, for the presence of CMV specific antibodies. 172 If this sample was positive (a titre of $>1:10$) no further samples were requested and the sample stored for further analysis if required. Those women seronegative for CMV antibody were retested at intervals to detect primary CMV infection (seroconversion).

As soon as possible after birth a throat swab was collected from all infants born at the three hospitals and cultured for CMV to detect congenital CMV infection. 7 A short form was completed by the midwife providing details of date of birth, birthweight, sex, gestation and any obvious malformation (Appendix 2). This form was returned to the laboratory with the throat swab. The information from the antenatal questionnaire, infant details and laboratory results of maternal serology and swab culture were all coded onto a single form (Appendix 3).

(ii) Diagnosis of congenital CMV infection

The throat swabs were collected into virus transport medium, within the first few days of life, and kept at 4°C prior to transport to the laboratory. Throat swab was chosen for screening as it has been shown to be effective 127 and is more appropriate for large-scale screening than the collection of urine samples. However, since no single method is 100% sensitive, urine and cord blood samples were also collected from those infants most at risk, that is, infants of mothers who experienced primary infection in pregnancy. In addition, cord blood samples were obtained from all babies born at one hospital and retained for testing when congenital infection was suspected.

In the laboratory, throat swabs were inoculated, in duplicate, into tubes containing tissue culture of human embryo lung cells, incubated at 40°C, and inspected at regular intervals for four weeks. CMV was identified by the appearance of typical cytopathic effect (CPE). Urine samples were first filtered and then inoculated direct into cell culture, in duplicate, as for throat swabs.

Cord blood samples were separated and the serum tested initially by CF test to confirm the presence of CMV IgG antibodies. If positive, the samples were further tested by Dr. P.D. Griffiths for CMV specific IgM antibodies by solid phase radioimmunoassay.

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A diagnosis of congenital infection was made when virus was isolated from either the throat swab or urine sample within three weeks of birth. 166 In addition, any infant with CMV specific IgM antibodies in cord blood in titre >1:100 was included as a case. 75 Infants transferred from other neonatal units were not

included in the study.

(iii) Definition of type of maternal infection

Congenital CMV may follow either primary maternal infection or recurrent infection. 194 Primary infection was diagnosed when CMV antibodies were acquired during pregnancy, and recurrent infection when CMV specific antibodies were present prior to pregnancy. However, the detection of antibodies at the first antenatal attendance does not exclude primary infection occurring between conception and this visit. As CMV specific IgM antibodies detected by RIA are consistently associated with recent primary infection and persist for up to 16 weeks, it is possible to distinguish primary from recurrent infection in seropositive women when they presented for antenatal care prior to the 16th week of pregnancy. 74,97,98

In the present study, the type of maternal infection was, therefore, classified as follows:

- I Confirmed primary infection :seroconversion during pregnancy
- II Presumed primary infection :CMV IgG and specific IgM antibody present in first antenatal serum sample
- III Confirmed recurrent infection:CMV specific antibody present prior to pregnancy
- IV Presumed recurrent infection :CMV IgG antibody present, but CMV specific IgM absent prior to the 16th week of pregnancy
- V Unclassifiable :CMV IgG antibody present, IgM absent at first antenatal attendance after the first 16 weeks of pregnancy

2. LONGITUDINAL STUDY OF THE CHILDREN WITH CONGENITAL CMV INFECTION

In this study all children who were diagnosed to have congenital CMV infection were followed up. As virus isolation may take up to three weeks, the diagnosis was often not made until the child was several weeks old and had been discharged from hospital.

Once the diagnosis was confirmed, the research paediatrician (PMP) contacted the consultant obstetrician and paediatrician and informed the general practitioner. With their consent, arrangements were then made to visit the family.

i) Initial visit and examination

Once the parents had been told the diagnosis and had consented to take part, details of paternal age, race and country of birth were collected together with details of the previous and family health of both parents (Appx 4). Particular enquiry was made into a family history of deafness and/or psychomotor delay. Opportunity was also taken to clarify any details on the Maternal Master Card which were ambiguous, particularly parental occupation. The mother was also asked further details of her pregnancy and in particular if she smoked or suffered from pre-eclampsia, antepartum haemorrhage or gestational diabetes melitis. She was also asked for details of labour and delivery, and about the progress of her infant in the neonatal period. These details were later confirmed by reference to hospital records.

Each infant was examined personally with measurement of occipitofrontal circumference (OFC) followed by a complete physical examination to detect eye defects, cataract, cardiovascular anomalies, rash, respiratory infection, abdominal masses, and, in particular, hepatosplenomegaly. Examination of the central nervous system included observation of the state of awareness, light reflex, ability to fix and follow, the presence of rooting, grasp and Moro reflexes, and symmetry of tone and movement. Finally, a urine sample was collected from each infant to monitor virus excretion.

ii) Review of maternal and neonatal records

The case records of all mothers and their babies were scrutinised to confirm infant gestation, problems during pregnancy, mode of delivery and, in particular, the reason for intervention if required. Measurements of birth weight, length, OFC, placental weight and Apgar scores were recorded, together with details of type and length of neonatal resuscitation and other neonatal

problems. In jaundiced infants, the maximum serum bilirubin measurement was recorded, and any treatment received. Any abnormality noted was recorded and the diagnosis on discharge was based on the paediatrician's assessment.

3. SELECTION OF CONTROLS

Since none of the features of congenital CMV are sufficiently specific to be synonymous with infection it was necessary to compare the progress of congenitally infected infants with a group of control children. As many of the problems which may result from CMV may be influenced by such variables as maternal race, age, parity, marital status, social class and infant sex, controls were matched for these factors. Controls were selected from within the study population, once congenital infection had been excluded, using the Maternal Master Cards completed at the antenatal clinic. The matching criteria were: maternal age within two years, parity (primigravidae or multigravidae,) marital status (married and cohabiting women were regarded as synonymous and single women whether unmarried, widowed, separated or divorced as single), race and country of birth. No information was available on paternal race at the time of control selection, so no attempt was made to match children of mixed race except by maternal race. Social class was matched on paternal occupation using the Registrar General's classification. 165 For single mothers who were not cohabiting their own social class was used for matching. If several possible controls were available who meet the criteria, other minor factors such as family size, country of birth and maternal occupation, and date of birth of the baby were used to obtain as closely matched controls as possible. No attempt was made to match for infant birthweight or gestation as these were factors which may be influenced by CMV.

Once selected, the mothers of control children were contacted after the general practitioner's permission had been sought. If agreeable, they were visited personally, at home, and told of the study procedure and invited to take part. Once permission was given, they were interviewed in exactly the same way as the parents of congenitally infected infants and the infants examined in an identical manner. The hospital records were also used to complete

the data. Following this initial visit which took place usually when the controls were about three months old the follow-up of both congenitally infected children and controls was identical. Since both parents were fully informed of the study it was not possible to examine the children blind.

4. SUBSEQUENT EXAMINATIONS

The initial assessment of cases was carried out at about six weeks of age. The children were then seen in the paediatric outpatient clinic at three, nine, 18, 24, and 36 months. As the control children were not seen until three months, the initial examination and three-month visit were combined. Any child who did not attend was seen at home, if possible, including those who moved out of London.

At each visit the parents were asked for an assessment of the child's general health since, the last visit, and specific enquiry was made into the frequency of upper and lower respiratory and ear infections. This was assessed using the parents' judgment: i.e. no infections, occasional infections and frequent infections. The severity of infections was assessed by the type of treatment required ranging from symptomatic measures at home to admission to hospital.

Enquiry was also made into the parents' opinion of the child's hearing and vision and the progress in development since the last visit. This was followed by a developmental assessment and completed by a general physical examination including measurements of height, weight and OFC. A urine sample was collected from each congenitally infected infant at each visit to monitor virus excretion.

5. DEVELOPMENTAL ASSESSMENT

i) STYCAR sequences

Since each child was seen at least five times in the first three years a method of developmental assessment was required which would be simple to perform and reproducible at different ages. As a physical examination and hearing assessment were also required,

the developmental assessment needed to be short and not involve the use of complex equipment. The Stycar sequences provided a descriptive analysis of the abilities of children up to five years. 181 This formed the basis of a method of assessment used in the National Childhood Encephalopathy study. 134 With the help of the late Dr. Sheridan the authors selected 151 cardinal items to cover the ages up to 36 months. 13 These items were divided into four major areas of development and scored to produce a numerical measure of the child's developmental level which could be compared with chronological age (Appx 5). This method of assessment was particularly appropriate for the present study, since it not only met all the conditions above, but also allowed comparison longitudinally and with other methods of assessment. There was no need to use external standardisation as the results would only be used to compare cases with controls.

ii) Griffiths Assessment

At two years a full measure of the child's developmental progress was obtained using the Ruth Griffiths developmental quotient. 78 This has been well standardised on preschool British children and correlates well with subsequent measures of intelligence.

iii) Stott Test of Motor Impairment

Items from the Stott test 209 were used to assess fine motor coordination at the 36 month visit. This was combined with the "Draw a Man test" 65 as an adjunct to the Stycar sequences. Children at this age respond poorly to timed tests, so a simple score system was used to judge the proficiency in each task. The "Draw a Man" score depended on the number of additions such as arms, legs and fingers added to a recognisable head. The tests and scoring system used are shown in Appendix 6

6. AUDIOLOGICAL ASSESSMENT

Each child received a full audiological assessment before the first birthday, and again at three years, to detect deterioration. Both these audiological assessments were carried out by a

paediatric audiologist.

The nine month assessment was done by distraction tests to voice and high and low tones. A tympanogram was used to assess middle ear function. At the three year visit, conditioning tests were used to voice and pure tones. If the child was cooperative these were repeated with headphones to produce a full audiogram. A Kendal toy test was also used to assess speech sound discrimination. The impedance tympanogram was repeated at this visit.

Any child who failed at these screening tests was seen subsequently to monitor the hearing levels. This allowed transient problems to be excluded and permanent neurological abnormalities to be monitored for any deterioration of hearing levels.

7. ASSESSMENT OF GROWTH

The growth of children with congenital CMV and controls was compared using population studies of normal children to provide normative data. Birth weights were compared with the data prepared by Thompson et al corrected for gestational age, sex and birth order. 217 Data from Usher and McClean on the length and head circumference, by gestation was used for comparison as equivalent data on a uniform British population is not available. 221

Standards of longitudinal growth in boys and girls were obtained for height from Tanner, Whitehouse and Takashi 215 and for head circumference from Tanner and Thomson. 214 All measurements were then converted to standard deviation scores so that they were directly comparable.

The standard deviation score (SDS) is a measure of the number of standard deviations the measurement is from the expected mean at each age. In a normal population, therefore, the mean SDS will be zero. SDS is calculated according to the formula $(x - \bar{x})/SD$ where x is the measurement, \bar{x} the mean (ie. 50th percentile for that age) and SD the standard deviation at that age. All calculations of age at the time of measurement were corrected for prematurity.

8. MATERNAL CHARACTERISTICS ASSOCIATED WITH AN INCREASED RISK OF CONGENITAL INFECTION

The maternal, racial and socioeconomic backgrounds of children with congenital CMV were compared with the population screened to ascertain whether congenital CMV was more prevalent in a particular subsection of the study population. The data of maternal characteristics is not yet complete for the full period of infant screening. The available data consisted of 6912 women who had delivered live births at Hospitals I and II over a two year period and a consecutive group of 1000 women who had live births at Hospital III. Infant screening took place between 1.3.80 and 30.6.83, for a period of 2.4 years at Hospital I, 3.3 years at Hospital II, and 3.25 years at Hospital III. Since the women attending each of the three hospitals were significantly different in terms of race and social background, the data cannot be combined without adjustment for the proportion of women attending each hospital, the different periods of maternal data available and differing periods of infant screening.

The data therefore were used to construct an estimated population similar to the actual population screened. Assumption was made that maternal characteristics of women attending each hospital remained constant throughout the screening period. The maternal data from each hospital was multiplied by the ratio of the period of infant screening to the period of available maternal data. The data from each hospital was then combined to form the "estimated population" reflecting the characteristics of the actual population screened, and directly comparable with the mothers of infants with congenital CMV.

The congenitally infected infants and the population data were modelled by group logistic regression using the GLIM statistical system in order to estimate prevalence rates and test for differences in prevalences between groups. 11

9. FAMILY STUDIES OF CHILDREN WITH CONGENITAL CMV

In order to determine whether infants with congenital or early acquired CMV were an important source of infection for their families, eight families were selected for more detailed study. In each family, samples of serum were collected from all adult members and tested for the presence of CMV antibody by CF test.

172 The seropositive samples were retested by RIA for CMV specific IgM to detect recent primary infection. 74 Thereafter each seronegative member of the family was retested at intervals to detect seroconversion. Urine and throat swab samples were collected from those members of the family who were seropositive and children in whom serological tests were felt to be unjustifiable. If virus was recovered from any contact the restrictive endonuclease typing was carried out of all virus isolates from that family to compare DNA structures and detect related strains. 56

10. ANALYSIS OF RESULTS.

The clinical, audiological and laboratory results were coded and stored using the computer facilities provided by the University of London central computer. Results were analysed using standard statistical tests available on the SPSS program. The complex multifactorial analyses of growth, developmental assessment, and population data was performed by Dr. A. Ades using logistic regression techniques. 11

RESULTS.

1. THE IDENTIFICATION OF CHILDREN WITH CONGENITAL CMV INFECTION

The screening of infants for congenital CMV infection took place between 1.3.80 and 30.6.83, and fifty infants were diagnosed. Overall 90% of infants were screened, the rates for each hospital being 93.5%, 90.5% and 83% respectively. The rate of screening was similar in low birth weight and premature infants and infants of normal birth weight.

The methods of diagnosis are tabulated in table 8. The throat swab was positive in 42 of 48 cases (87.5%) and was the sole method of diagnosis in 18 cases. In the remaining 30 cases cord blood samples and/or urine samples were also available to confirm the diagnosis. In one case the initial urine sample was negative for CMV when the throat swab was positive, but further samples of urine contained virus. In total, 24 of 25 (96%) urine samples were positive by virus culture. Cord blood samples were available in 19 cases. When tested for CMV specific IgM by RIA, 16 (84%) had a titre $>1:100$ but in three cases the titre was $<1:50$ and this was regarded as negative, although virus was isolated from throat swab and/or urine.

In two cases throat swabs were not collected, but urine and cord blood samples were available, and both were positive.

In summary, therefore, urine culture was most sensitive as a method of diagnosis with a false negative rate of 4%, while throat swab culture and cord blood IgM assay have false negative rates of 12.5% and 16% respectively.

2. TYPE AND TIMING OF MATERNAL CMV INFECTIONS

Table 9. shows the type of maternal CMV infection and when it occurred. The mothers of 18 congenitally infected children seroconverted during their pregnancy and the mothers of 29 were seropositive at their first antenatal attendance. No serological data was available in three cases.

TABLE 8.
Method of diagnosis of 50 children with congenital CMV infection.

Samples collected	n.	CMV Isolated(%)		CMV Specific IgM >1:100
		T.S.	Urine	
T.S. + Urine + IgM	10	8	10	7
T.S. + Urine	13	13	12	-
T.S. + IgM	7	3	-	7
T.S. only	18	18	-	-
Urine + IgM	2	-	2	2
<hr/>				
Total	48	25	19	50
		42(87)	24(96)	16(84)

T.S. = Throat swab

TABLE 9.
Type and timing of maternal infection in 50 cases of congenital
CMV

Type of maternal infection	n.(%)	Gestation (weeks)			NK
		<16	16-27	28-40	
Primary I (Seroconversion)	18(36)	1	5	10	2
II (Presumptive)	14(28)	8	2	4	-
Recurrent III (Confirmed)	3(6)	-	-	-	3
IV (Presumptive)	7(14)	-	-	-	7
Not Known Va (Not Classifiable)	5(10)	-	-	-	5
Vb (No Serology)	3(6)	-	-	-	3
<hr style="border-top: 1px dashed black;"/>					
Total	50(100)	9	7	14	20

When the 29 initially seropositive samples were further tested for the presence of CMV specific IgM, 14 were positive and presumed to follow recent primary infections. No specific IgM was present in the remaining 15 cases. In three of these, sera from a previous pregnancy was available and found to be positive for CMV antibody, confirming that recurrent infection had occurred and was transmitted in utero. In the seven cases who booked within 16 weeks of their last menstrual period, the absence of CMV specific IgM excluded recent primary infection, and maternal infection was presumed to be recurrent. In the remaining five cases who booked for antenatal care after 16 weeks no specific IgM was detected, but early primary infection in pregnancy could not be excluded and the type of maternal infection could not be defined.

In all but two of 32, primary maternal infections (both confirmed and presumed) it was possible to assess the trimester in which infection had occurred, from the dates of seroconversion and the persistence of CMV specific IgM. In the two cases for which this was not possible, the first antenatal blood sample was seronegative at 9 and 15 weeks respectively, and cord blood was positive, confirming seroconversion but no follow-up maternal serum sample was available to test for CMV specific IgM. Nine (28%) of primary infections occurred before 16 weeks, seven (21%) between 16 and 27 weeks and 14 (43%) at 28 weeks or after.

3. SELECTION OF CONTROL CHILDREN.

Two control children were selected for each case. Suitable controls were selected for all but two cases. The two children for whom no controls were selected live permanently abroad and it was thought inappropriate to attempt to select controls. Further analysis of the two groups (Table 10.) showed no significant differences on the matched criteria of maternal age, race, country of origin, marital status, family size, social class and infant sex. Although it was not possible to match for paternal race and thus infant race, the number of mixed race infants was similar in both groups.

TABLE 10.
Comparison between children with congenital CMV and control children

<u>Maternal characteristics</u>		<u>Congenital CMV</u> n.=50	<u>Controls</u> n.=97
Age(years)	mean(SD)	24.06(5.8)	24.13(5.8)
Parity	mean(SD)	0.54(1.1)	0.48(1.0)
Marital status	Single	23	40
	Married	24	51
	Cohabiting	3	6
Social class:	Non-manual	13 (23)	32 (56)
Paternal(House)	Manual	9 (11)	22 (26)
	Other	5 (16)	3 (16)
	Single parent	23	40
Race	Caucasian	32	63
	Asian	4	8
	Black	14	26
<u>Infant characteristics.</u>			
Sex	Male	26	52
	Female	24	45
Race	Caucasian	29	56
	Asian	3	8
	Black	13	26
	Mixed	5	7

*Social class - Other = H.M.Forces,students,not known.
(House) = Social class of householder including single parents.

SD = Standard deviation.

4. CHILDREN WITH CONGENITAL CMV.

i) Prenatal problems and fetal growth

There was no difference in the frequency of hypertension, pre-eclampsia, antepartum haemorrhage or gestational diabetes during pregnancy in mothers of congenital infected infants and controls. The proportion of smokers was similar. The rate of premature delivery was the same in cases and controls, and there was no difference in type of delivery. None of the mothers of congenitally infected infants reported symptoms suggestive of CMV infection. Travel abroad was significantly more frequent ($P < 0.02$) though most stayed within Europe (Table 11.).

The mean gestational age, birth weight and length of congenitally infected infants were not significantly different for controls (Table 12). The mean head circumference of congenitally infected infants was slightly lower than in controls with greater variance, but this was not significant. When account was taken of gestation, intrauterine growth retardation and microcephaly ($\text{OFC} < 3\text{rd percentile}$) were no more frequent in cases compared with controls.

ii) Problems noted in the neonatal period (Table 13.).

One child with congenital infection was noted to have a major congenital abnormality (ventricular septal defect). One control child had a similar abnormality. Two other controls had abnormalities, one unilateral ptosis, the other an accessory digit. There was no evidence of increased fetal hypoxia in congenitally infected infants as judged by the Apgar score, or number admitted to SCBU than controls. The frequency of jaundice and maximum bilirubin level were similar and hepatomegaly was noted in only one infant with congenital CMV.

Two children presented as suspected cases of intrauterine infection and both had CNS symptoms. The first suffered from severe intrauterine growth retardation, severe microcephaly, and dystonia. The second child had hepatomegaly, hypotonia and stridor, and required tube feeding. A third child, in whom congenital infection was not clinically suspected, developed respiratory distress on the third day due to pneumonitis,

TABLE 11.
Maternal factors in children with congenital CMV and controls.

	CMV n.50	Controls n.97	p Value
Smokers	12	12	NS
Hypertension	2	14	NS
Antepartum haemorrhage	1	1	NS
Gestational diabetes	1	3	NS
Travel abroad	8	5	p<0.02
Assisted delivery	16	21	NS

NS = Not significant.

TABLE 12.
Intrauterine growth of children with congenital CMV and controls.

	CMV n.50 Mean (SD)	Controls n.97 Mean (SD)	p Value
Birth weight (kgs)	3.21(0.68)	3.22(0.48)	0.93
O.F.C. (cms.)	33.8 (2.2)	34.3 (1.5)	0.12
Birth length (cms.)	51.6 (4.8)	51.75(3.1)	0.78
Gestation (weeks)	39.3 (1.8)	39.3 (1.6)	0.83
n.Small for gestational age.	5	3	0.09
n.O.F.C.<3rd percentile	11	11	0.1>p>0.05

O.F.C. = Occipitofrontal circumference

TABLE 13.
Features noted in the neonatal period in children with congenital
CMV and controls.

	CMV n.50	Controls n.97	p.Value
n.Admitted to S.C.B.U.	5	6	0.18
Mean Apgar 5 mins	9.75	9.63	0.26
Mean max bilirubin (umols/l)	227	226	0.97
Hepatomegaly	1*	0	0.34
CNS problems	2*	0	0.57
Congenital defects	1+	3	0.60
Respiratory problems	3*+	0	0.04
Other problems	4+	4	0.20
Total n. problems	7	7	N.S.

*,+ = Two children with multiple problems.

associated with a small VSD, and in addition had the characteristic appearances of fetal alcohol syndrome. Recovery was also complicated by necrotising enterocolitis. The respiratory illness was likely to be due to CMV, as no other pathogen was isolated, the chest X-ray appearances showed bilateral infiltrates and there was no response to antibiotic therapy. Two other cases had respiratory problems, one noted above with stridor related to hypotonia, and another with transient tachypnoea of the newborn.

A wide range of other problems occurred in cases and controls. Two infants with congenital CMV suffered from localised candida infections and one had conjunctivitis. One control child was admitted to SCBU for treatment of hypothermia, two developed jaundice; one due to glucose-6-phosphate dehydrogenase deficiency, the other ABO incompatibility and one suffered from positional talipes.

In summary only three (6%) cases had significant symptoms in the neonatal period. No child had splenomegaly, rash or thrombocytopaenia.

5. FOLLOW-UP OF CHILDREN WITH CONGENITAL CMV AND CONTROLS

All but one of the 50 cases has been followed up personally, and, for the remaining child, who lives abroad, several reports are available from the child's personal physician. To date, (1.6.84,) none of the cases or controls have died and none of the cases have withdrawn from the study. Seven controls have withdrawn from the study at ages ranging from 5-32 months. However, all but two completed the 9-month assessment, and one completed the two-year assessment. The number of children seen at each age is summarised in Table 14.

Initial examination

The initial assessment provided an opportunity to confirm the abnormalities noted at birth and to examine the child in detail to identify previously unrecognised or new abnormalities. The number of children with abnormalities is noted in Table 15, with a list of new abnormalities noted at each assessment. There was a wide range of abnormalities in both the children with CMV and controls. Two distinct problems were particularly apparent in children with



TABLE 14.
Follow-up of children with congenital CMV and controls,
number examined at each age.

Age (months)	3	9	18	24	36
CMV n.50	49	47	30	29	18
Controls n.97	97	90	65	54	34

TABLE 15.
Children with CMV and controls; abnormalities noted at each examination.

Age(mths.)	<u>Congenital CMV</u>		<u>Controls</u>	
	n.abN/ n.total	New abnormalities	n.abN/ n.total	New abnormalities
3	8/49	2 hepatosplenomegaly 6 pneumonitis 1 umbilical hernia 1 candidiasis	9/97	3 umbilical herniae 1 bronchiolitis 1 2x ptosis +G6PD Def 1 craniotabes 1 syndactyly 1 hydrocoele
9	4/47	2 spastic- quadraplegia + epilepsy 1 optic atrophy	5/90	2 wheezy bronchitis
18	4/30	1 failure to thrive 1 wheezy bronchitis	4/65	1 macrocephaly + speech delay 1 asthma
24	3/29	nil	2/54	1 umbilical hernia
36	2/18	nil	1/34	nil

n.abN :number abnormal

G6PD Def :Glucose-6-phosphate dehydrogenase deficiency.

CMV in the first four months, ie. hepatosplenomegaly and interstitial pneumonitis.

Hepatosplenomegaly

Only one child had hepatomegaly in the neonatal period. However, two further children developed hepatomegaly in association with splenomegaly after the neonatal period. One of these children had had pneumonitis as a neonate, but, at the age of three months, when the respiratory problem was improving, transient hepatosplenomegaly was noted. The second child was admitted to hospital at four months with the acute onset of a rash described as "erythema multiforme" associated with hepatosplenomegaly. The rash subsided quickly but asymptomatic hepatosplenomegaly persisted until the age of 18 months with no recurrence of the rash.

Interestingly, one control infant had a non-specific illness at the age of 9 months characterised by generalised lymphadenopathy and hepatosplenomegaly. Infectious mononucleosis due to CMV and EB virus were excluded and resolution took place spontaneously over one month.

Respiratory Complications.

Six congenitally infected infants presented with respiratory symptoms, five at or before three months and one at four months. All were characterised by tachypnoea, hyperinflation and non-specific changes on chest X-ray. None was severely ill and only one required hospital admission. In all six instances CMV was cultured from pharyngeal aspirate as well as urine samples during the acute phase of the illness. Bacterial culture, viral culture for adenovirus, RSV, influenza and parainfluenza and serological titres for mycoplasma were all negative. In one child C.trachomatis was also isolated. The acute illness lasted from one week to one month in duration and the pharyngeal aspirate became negative, for CMV culture, with resolution of symptoms in all cases despite continued excretion of virus in the urine. Two children have continued to have intermittent respiratory problems up to one year of age.

The pattern of illness and the absence, except in one case, of an alternative pathogen, suggests that CMV was acting in these six children as a primary pathogen. The frequency of respiratory disease in congenitally infected infants was highly significant ($p=0.0001$).

One further case had a respiratory illness suggestive of B.Pertussis infection. Unfortunately previous antibiotic therapy did not permit bacterial isolation but pharyngeal aspirate was negative on CMV culture. One control child has had a number of episodes of "bronchitis" treated by her general practitioner but was not investigated.

Nine-month Assessment and Hearing Tests

Major delay in psychomotor development and neurological abnormality were apparent in two children with CMV at the nine-month assessment. Both had spastic quadriplegia. In one of these neurological abnormalities were suspected at birth and spastic quadriplegia and severe mental retardation with severe microcephaly confirmed in the first six months of life. She also has frequent fits and bilateral optic atrophy. Electrocochleography confirmed normal hearing.

The second child was SFGA (Bwt 2.0 kg OFC 28 cm) but otherwise normal at birth. Initial developmental progress appeared normal but he was not sitting by nine months and manipulation was immature. Physical examination revealed mild spastic quadriplegia. Fundoscopy was normal and skull X-ray revealed no evidence of intracranial calcification. He also now has frequent fits.

Hearing Assessments

Full hearing assessments have been carried out on 47 cases and 88 controls. In addition, the two cases who live abroad have been tested by their personal paediatricians; one has conductive hearing loss and the other has normal hearing. The parents of one congenitally infected child refused to attend for hearing assessment, but there is no clinical suspicion of deafness. Among the controls, six have withdrawn from the study, and three were unable to attend for hearing assessments. These children were seen at home and clinically hearing was normal. The results of the hearing assessments are shown in Table 16. Five cases (11%) have sensorineural deafness, three (6.5%) are bilateral and hearing aids have been prescribed. In two of these, there was also a conductive element. In one case with bilateral sensorineural deafness, neurological examination including fundoscopy was normal, despite the presence of symptoms in the neonatal period

TABLE 16.

Hearing assessments in children with congenital CMV and controls.

Age (months)		Congenital CMV n.50		Controls n.97	
		9	36	9	36
n.tested		47	14	88	33
Normal		27	14	55	26
Sensorineural Deafness(SN)	Bilateral:	1	-	-	-
	Unilateral:	2	-	-	-
Mixed (SN + CD)		2	-	-	-
Conductive Deafness(CD)	Bilateral:	15	-	21	4
	Unilateral:	-	-	6	3
Equivocal		-	-	6	-

(hepatomegaly and hypotonia). In a second child, spastic quadriplegia is also present. In this child subsequent testing revealed resolution of the conductive element but despite this the hearing thresholds deteriorated to 90 dB bilaterally between nine and 18 months. It has not been possible in the two children with suspected unilateral deafness to perform full audiometric testing with masking, using earphones, but this will be done at the four year examination to ascertain the true thresholds more accurately. Sensorineural deafness was not present in any of the controls.

Conductive hearing loss was common in both cases and controls. At the nine-month examination 17 cases (37%) had bilateral conductive loss (including two mixed) compared with 27 controls (31%), 21 bilateral and six unilateral, conductive loss. This difference was not significant ($\chi^2 = 0.246$). To date 13 cases and 21 controls have been retested. This has confirmed bilateral sensorineural deafness in all three cases, conductive deafness in five, a further five with previous conductive deafness have resolved and five await retesting. Amongst controls conductive deafness has persisted in nine and has resolved in 11 whereas seven await retesting.

Further Hearing Assessments at 36 Months

Only one child showed a deterioration in hearing levels after the first hearing assessment. This child has not yet reached 36 months but repeated tests show a stabilisation of hearing thresholds from 18 months. None of 14 cases and seven of 33 controls had persistent conductive hearing loss at 36 months. Not all children are sufficiently mature at 36 months for headphone audiometry so further cases of unilateral deafness cannot be excluded. However, no sensorineural deafness has been detected since the first audiological assessment.

18 Month Assessment

Few new problems became apparent after one year of age (table 15). One child, who had pneumonitis, continues to have episodes of wheezy bronchitis. Delay in psychomotor development became apparent, for the first time, in two older children. One child, with congenital CMV, already identified at risk due to fetal alcohol syndrome, continues to thrive poorly with height, weight and OFC all below the third percentile. This child has mild

psychomotor delay despite normal neurological examination. It is possible that fetal alcohol syndrome may account for this child's problems and CMV may be an incidental finding. The other, a control infant, with an OFC above the 97th percentile, also has mild psychomotor delay and particularly poor language development.

Assessment at 36 Months

No further abnormalities of physical or intellectual development have been detected in those children who have reached 36 months and there has been no evidence of progressive disease amongst the cases (table 15).

6.MEASUREMENTS OF INTELLECTUAL PROGRESS IN CHILDREN WITH CMV COMPARED WITH CONTROLS.

Griffiths assessment at 24 months

To date the developmental quotient (DQ) of 29 cases and 53 controls have been measured. Intellectual impairment was defined as a DQ more than 2SDs below the mean score of control children. Four cases and two controls fell into this category and this difference was not significant ($P=0.09$ Fisher's exact test.)

The mean score of cases and controls for both overall DQ and the five subscores are shown in Table 17. While the scores for cases are lower than for controls this is not significant ($P=0.08$).

All children in the study, both with congenital CMV and controls, were further divided up to exclude those with permanent neurological sequelae on follow-up. All five children with neurological sequelae were congenitally infected and included the two children with spastic quadriplegia, one with bilateral and two with unilateral sensorineural deafness. Comparison between controls and congenitally infected infants, with no sequelae, shows no evidence of an effect of CMV on intellect at two years in the absence of neurological abnormality. The subscores show no specific area of development has been affected. These data were subjected to logistic regression analysis to separate the effects of CMV from the effect of social class and other matching variables. The independent effect of each variable, on an individual child's DQ score, compared with controls, is shown in Table 18. This confirms that there was no difference in

TABLE 17.

Mean Developmental Quotient of children with congenital CMV and controls (SD).

	Controls n.53	CMV n.29	CMV n.24 No sequelae	CMV n.5 Neurological sequelae
Total	101.8 (8.3)	95.0 (21.7)	101.2 (10.1)	69.0 (40.1) p 0.001
Locomotor	106.3 (8.3)	101.4 (23.3)	106.3 (14.2)	78.0 (42.6) p 0.01
Hearing and speech	98.6 (14.5)	91.8 (25.1)	98.4 (17.8)	59.8 (32.5) p 0.001
Eye-hand coordination	97.2 (9.5)	90.5 (23.2)	97.2 (10.4)	62.2 (39.9) p 0.001
Social	106.4 (13.3)	100.4 (23.6)	105.9 (13.8)	74.4 (42.1) p 0.004
Performance	99.5 (9.4)	96.4 (24.2)	102.2 (12.2)	70.0 (45.4) p 0.005

p ; significance level CMV neurological sequelae compared with controls.

TABLE 18.

The effect on a child's developmental quotient of individual variables.*

		Effect on D.Q.	95% C.I.	p.
CMV no sequelae		+ 2.1	5.1	NS
CMV neurological sequelae		-35.6	11.4	<0.0001
Maternal age	20-24	- 1.7	6.5	} NS
	25-29	+ 0.9	8.6	
	30+	+ 3.8	9.4	
Social class	manual	- 6.6	6.3	} 0.009
	other	- 9.0	6.3	
Race	Asian	- 0.3	9.2	} 0.06
	Black	- 5.9	6.1	
Parity	para 1+	- 5.2	5.5	0.06
Marital status	married	- 0.8	6.5	NS
Infant sex	male	- 0.8	6.6	NS

*Comparison assumes DQ = 100 for female controls of mothers <20 yrs, non-manual social class, white race, para 0, single marital status.

C.I. Confidence interval

performance of children with uncomplicated CMV compared with controls. It also highlights the significant effect of social class irrespective of the presence of congenital infection and the lesser effects of race and increasing family size on developmental achievements.

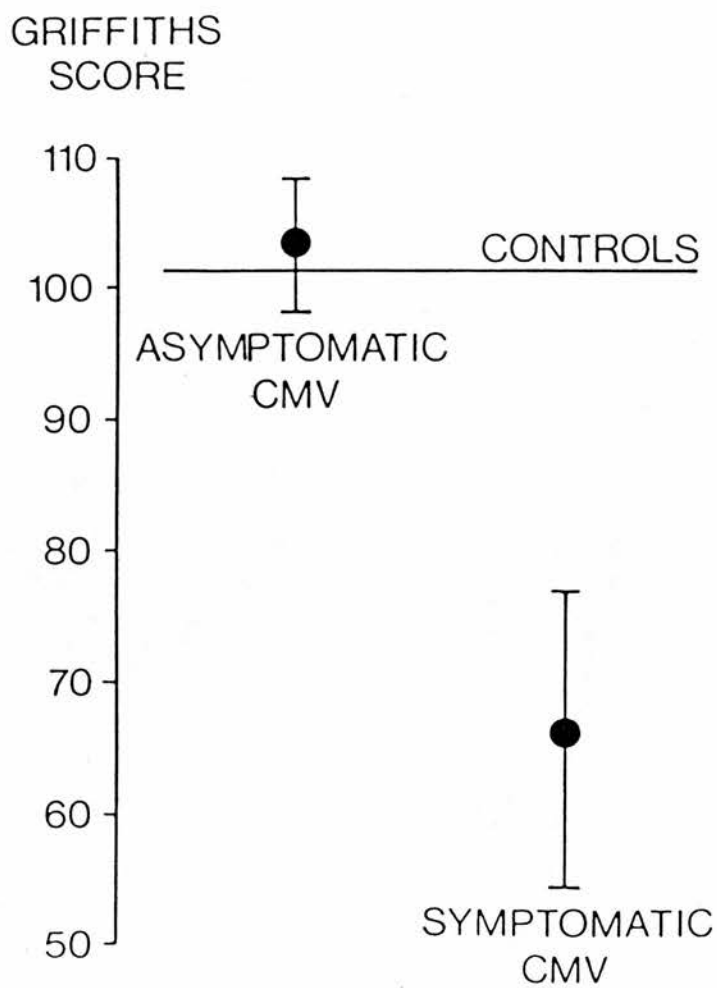
Children with neurological sequelae, as defined above, have a significantly lower mean DQ compared to controls both in total score and in subscores (Table 17) suggesting either that these children suffer also from intellectual impairment or their motor and sensory disabilities interfere with their ability to perform the test. The two most severely affected children, both with spastic quadriplegia, performed very poorly. Two children with deafness (one bilateral and one unilateral) had normal overall scores, but both had reduced hearing and speech scores (84 and 88 respectively.) Logistic regression (fig.2) confirms that the poor performance in this group was due to the presence of congenital CMV and not due to associated factors such as social class as there were no interactions between variables in the regression analysis ($P > 0.2$)

In the children who had intellectual impairment (ie. $DQ > 2$ SD below mean of controls) three cases came from the neurological sequelae group. The other congenitally affected child had fetal alcohol syndrome but no specific neurological deficit. One of the control children in this group was noted to have macrocephaly and may, on further detailed assessment by a speech therapist, have a specific language disorder. The other control who performed poorly had severe behaviour problems and associated language delay.

Stott Test of Motor Coordination

To assess the abilities of the children in fine motor manipulation, items from the Stott test of motor impairment were used, with the "Draw a Man" test, to identify children with "soft" neurological defects, such as clumsiness, at the 36 month assessment.

The items from the "sub-five" years test were used to assess the effect of CMV on motor co-ordination. The first two items (Appx V) were found to be too inconsistently performed by three-year-olds to be reproducible or comparable. When the remaining items were combined with a "Draw a Man" test the total



Adjusted mean Griffiths Developmental Quotient of children with asymptomatic (no sequelae) and symptomatic (neurological sequelae) congenital CMV compared with mean of controls.

FIG 2.

possible score was 25. Thirteen cases and 26 controls have now completed this test, and there is no significant difference in the mean scores of both groups (Table 19). Although the number assessed is still small this suggests no increase in clumsiness in the CMV group and suggests that minimal brain damage is not occurring in this area of motor skills.

Use of the Stycar Sequence

The Stycar sequences were initially used, as described by Dr. Sheridan, as a descriptive analysis of the abilities of babies to identify those at risk of intellectual, motor or sensory defects. The attempts to score the sequences did not begin until many of the children had reached two years. The tests were found to be satisfactory to score. They were simple to perform, required only minimal equipment and could be performed at home. The sensitivity and specificity have not been determined in a normal population. The results of the tests in controls are plotted on fig.3. This shows that children scored consistently along a quadratic axis which has been plotted. Fig. 4 shows how the children with CMV perform compared with the mean of controls. This suggests there is no difference between the scores achieved by cases and controls. This is confirmed when the results were subjected to a logistic regression to remove the effects of age and variation due to matching variables (Table 20). However, the number of children with neurological sequelae (six) assessed by this method was small and so a significant difference between those with neurological sequelae and those who escaped sequelae was only apparent at 18 months.

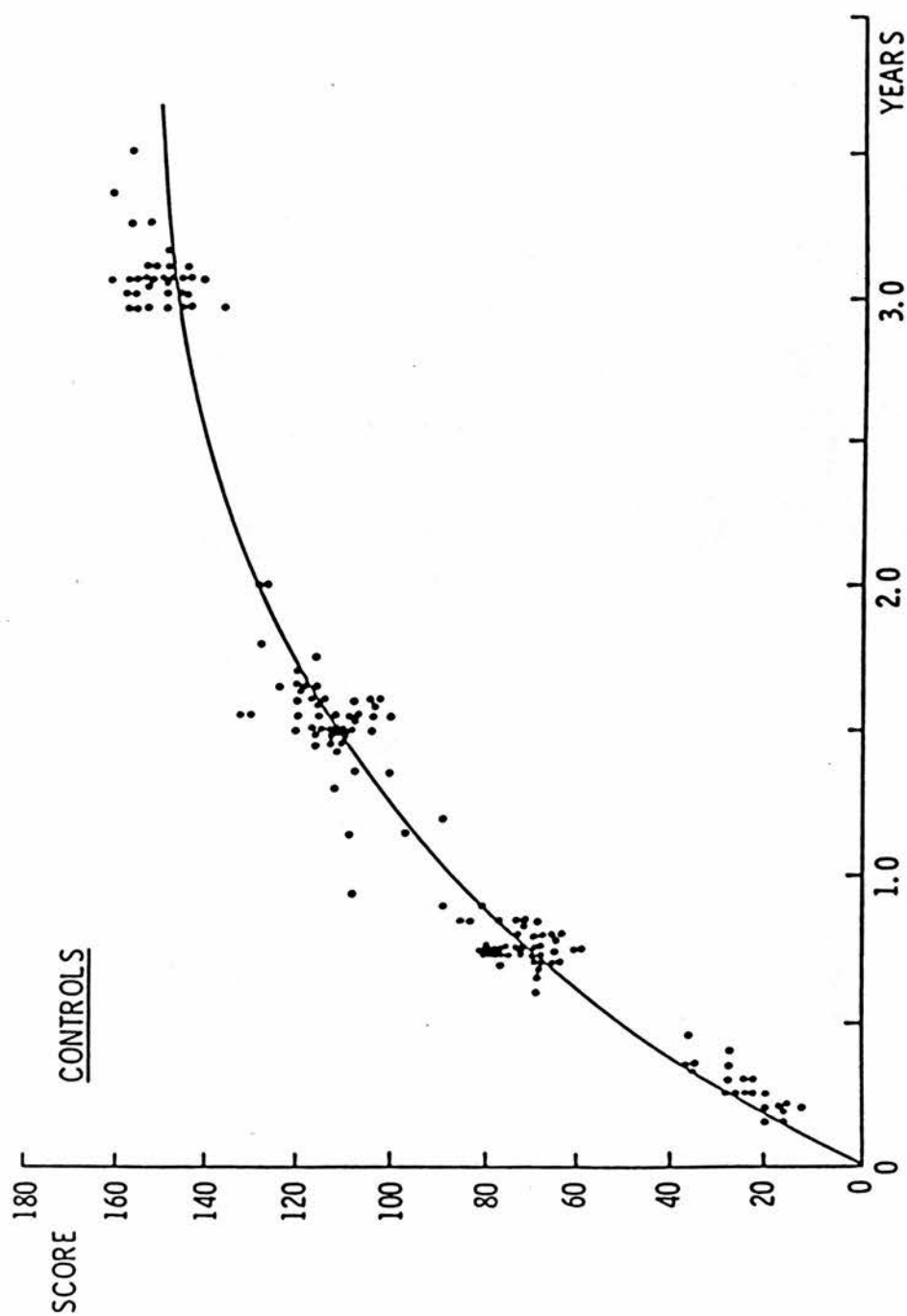
When this method of assessment is compared with the Griffiths test at 24 months, of the six in whom the Griffiths score fell below 2 SD from the mean of controls, one was not tested by the Stycar method, three scored poorly (one spastic quadriplegia at 18 months, one fetal alcohol syndrome and one macrocephaly at 36 months). Two had normal scores. One of these was assessed by Stycar at nine and 18 months when his behaviour problem was not so apparent as at 24 months when the DQ was performed. The second scored poorly in the Griffiths assessment but when examined at 36 months had made good progress and was clinically developing normally.

TABLE 19.

Tests of motor coordination in children with congenital CMV and controls.

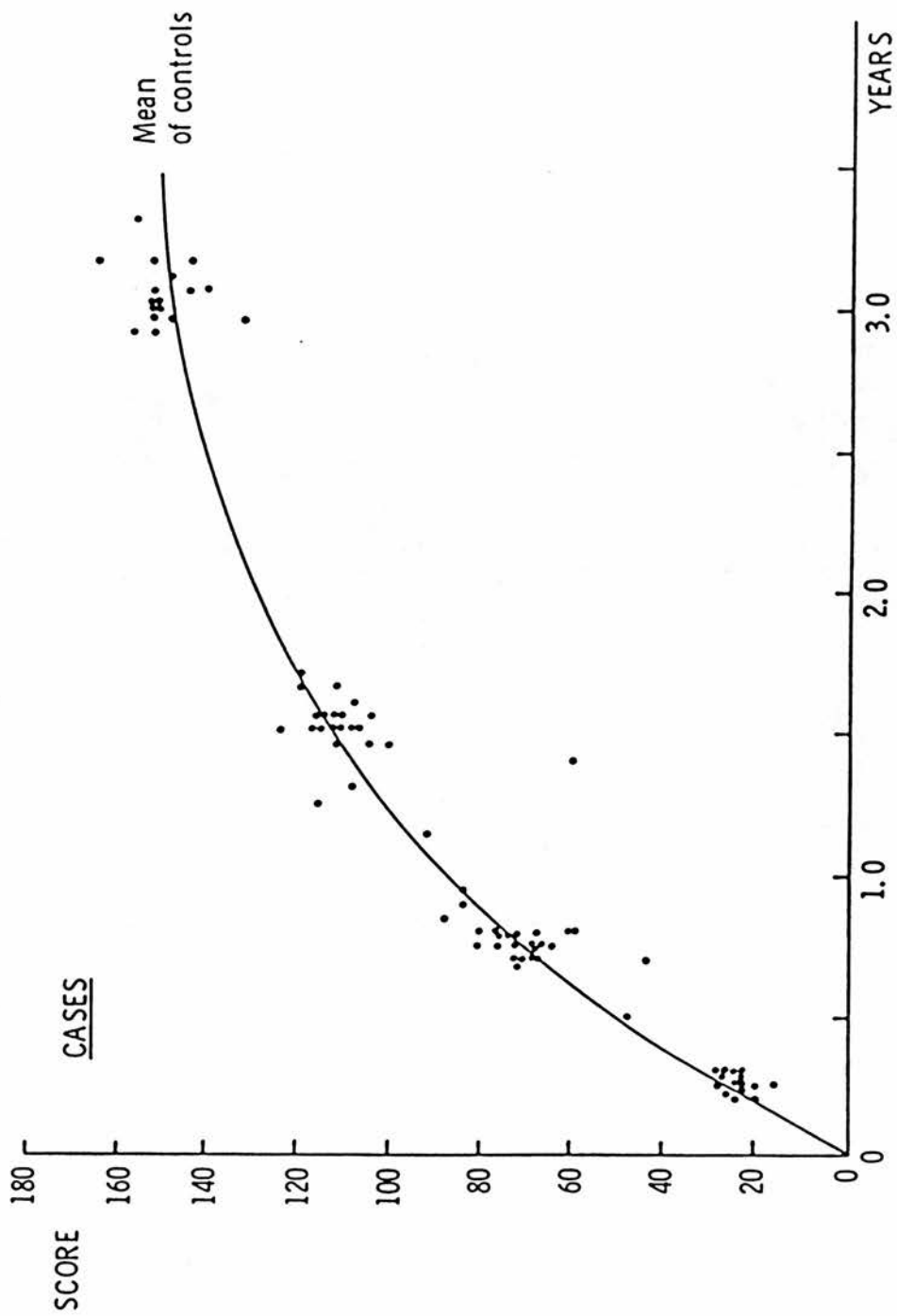
	Mean	SD
CMV no sequelae (n.13)	19.69	3.42
Controls (n.26)	19.73	1.78

p.=0.97



STYCAR test scores on control children.

FIG 3.



STYCAR test scores of children with congenital CMV compared with the mean of controls.
FIG 4.

TABLE 20.

Developmental testing using the Stycar scoring sytem;
mean difference from controls.

Age (months)	n.	CMV no sequelae (SD)	n.	CMV neurological sequelae (SD)
3	15	-1.51 (1.7)	1	0.05 (4.4)
9	23	0.88 (2.0)	2	- 7.23 (5.5)
18	18	-0.51 (2.2)	3	-19.63 (4.8)
36	15	-0.42 (1.8)	2	1.24 (4.8)

The Stycar sequence scoring system, therefore, while less sensitive than the Griffiths test, was a useful adjunct to the clinical examination, and was very adaptable to situations where the Griffiths test was less appropriate. However, the Griffiths assessment was required to document the abilities of the children in a reproducible and consistent manner. It was also required to assess the abilities of the children in specific areas, using the subscores, so that the particular areas of concern such as language could be highlighted. The Griffiths assessment will also be used to compare with IQ tests at five years to assess if children appear to have a "fall-off" in intellectual development with age as a result of CMV.

7. ASSESSMENT OF INTRAUTERINE AND LONGITUDINAL GROWTH AND RELATIONSHIP TO OUTCOME.

As shown in Table 12 no effect of CMV was demonstrated on intrauterine growth. This analysis did not, however, correct for factors such as gestation, birth order, maternal social class or race. The data on intrauterine growth was combined with measurements of postnatal growth to examine the effect of CMV, in more detail allowing for these factors, and in particular to assess if growth retardation was a feature of CMV alone and if it was a predictor of future outcome. The congenitally infected infants were divided, as in the Griffiths analysis, into those with or without permanent neurological sequelae on follow-up. The standard deviation scores of both groups of congenitally infected infants and controls for weight, head circumference and length at birth, and height and head circumference at three, nine, 18, 24 and 36 months are shown in Table 21.

The variability in the recorded measurements of birth length was extremely wide and is probably due to difficulties in performing measurements of this type in the labour ward where no stadiometer was available. In consequence measures greater than 3 SD from the mean in each group were excluded from the analysis of birth length. Inspection of these data suggest that CMV, which does not result in neurological sequelae, has no consistent effect on both intrauterine and postnatal growth. However, since there

TABLE 21.

Standard deviation score (SDS) of weight, length and head circumference (HC) of congenital CMV and controls.

Age (months)	<u>Controls</u>			<u>Congenital CMV</u>					
				no sequelae			neurological sequelae		
	n.	SDS	SD	n.	SDS	SD	n.	SDS	SD
<u>Weight</u>									
<u>Birth</u>	97	-0.17	0.98	44	-0.02	1.20	6	-1.29	2.09
<u>Length</u>									
<u>Birth</u>	94	0.73	1.48	36	0.44	1.33	4	-0.72	2.27
3	9	0.34	1.36	23	0.22	1.09	3	-0.56	2.28
9	75	0.07	1.33	36	-0.22	1.45	6	-1.03	1.87
18	60	0.04	0.95	23	-0.74	1.35	5	-1.28	1.89
24	38	0.40	1.06	20	-0.32	1.28	3	-1.83	1.71
36	31	0.40	1.01	15	-0.18	1.26	2	-0.13	2.24
<u>Head Circumference</u>									
<u>Birth</u>	96	0.38	1.16	44	0.45	1.35	6	-2.69	2.08
3	29	0.44	1.08	26	0.58	0.99	3	0.0	0.40
9	85	0.39	1.03	40	0.25	1.30	6	-1.74	2.47
18	62	0.33	1.19	25	0.13	1.20	5	-2.11	2.77
24	40	0.49	1.07	21	0.58	1.70	2	-4.75	4.89
36	31	0.05	1.32	16	-0.14	1.68	2	-1.05	0.58

were wide variations in maternal factors such as race and social class which could not be corrected for from normative data it is possible that real differences may not be detected by analysis of such data by simple unmatched T tests. These data were therefore subjected to logistic regression analysis to correct for maternal age, race, social class, parity, marital status and infant sex. By this measure the difference in SDS between CMV cases in both groups and controls could be calculated and 95% confidence limits and significance levels presented.

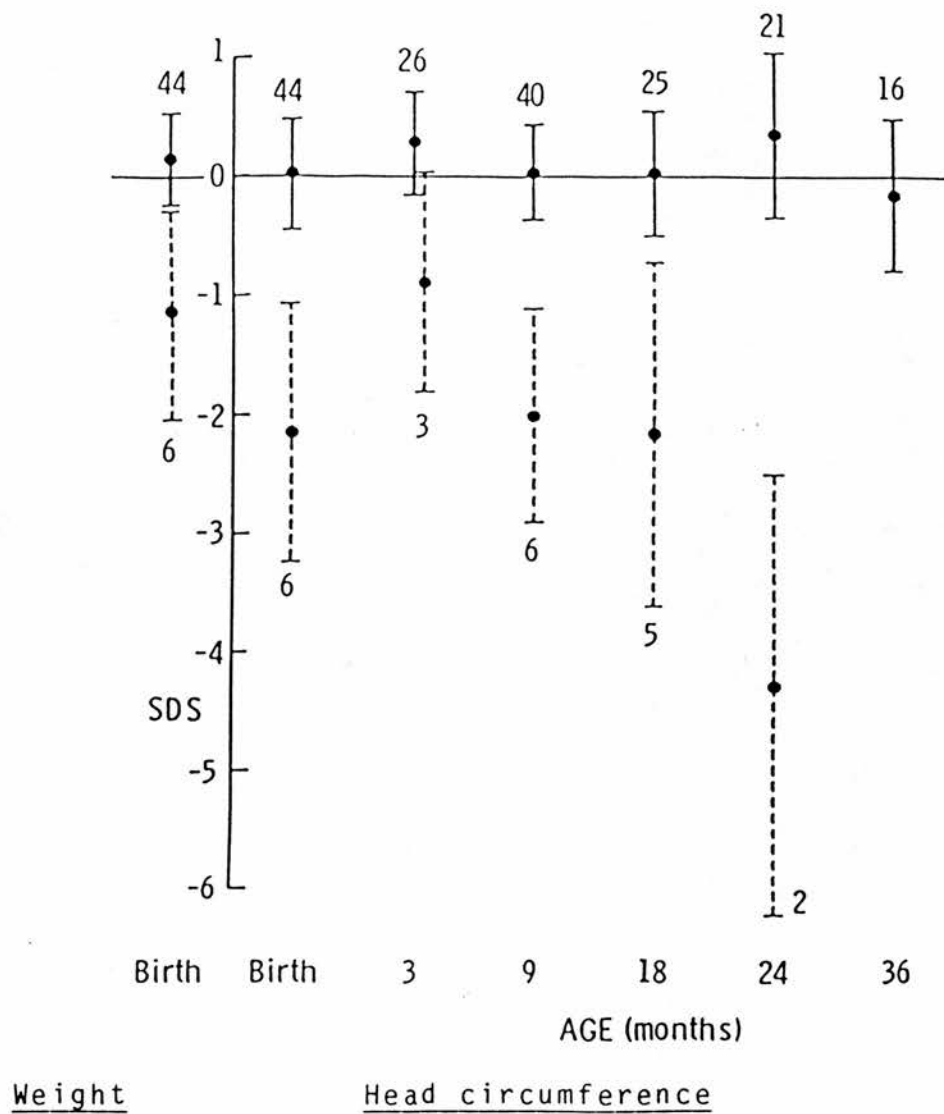
Birth Weight and Head Circumference.

The results for birth weight and head circumference are shown in fig.5 and for length in fig.6. It will be seen that there is no difference in the birth weight of children with CMV, and no subsequent sequelae, compared with controls. Similarly there is no significant difference in head circumference of congenitally infected infants from controls at birth or subsequently, with no evidence of failure of head growth in older children.

Children with neurological sequelae, as a result of CMV, however, are of significantly lower birth weight ($P=0.01$). All six children in this group had head circumference measured at birth, at nine months, and five at 18 months, and at each age there was a highly significant reduction in head circumference ($P<0.0001$). Only three children were measured at three months and a further two children measured at 24 months. These results therefore probably distort the true picture and, if excluded, there is no evidence of either "catch up" growth or progressive failure of head growth.

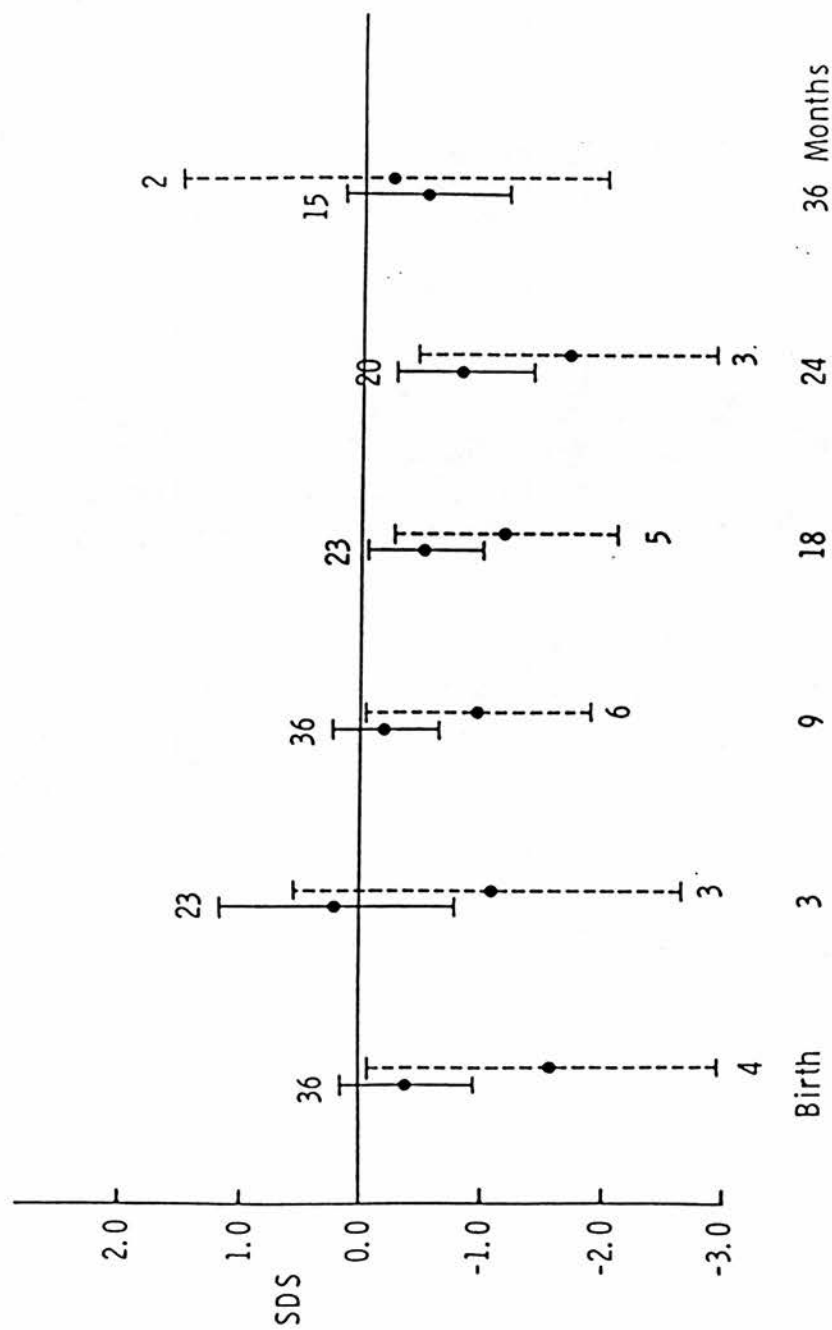
Somatic Growth.

There was no significant difference in the birth length and height at three and nine months between children with CMV with no sequelae and controls (fig.5.) However, there is a trend for the growth to fall behind controls which becomes significant at 18 and 24 months ($P=0.04$ and 0.006 respectively) but not confirmed at 36 months. It is possible therefore that these infants are not thriving as well as expected and this may be an effect of CMV. It will be interesting to see if this effect is confirmed as more children mature and so have measurements at all ages.



Adjusted mean Standard Deviation Score of head circumference and birth weight of children with congenital CMV with no sequelae (I) and neurological sequelae (II) compared with the mean of controls.

FIG 5.



Adjusted mean Standard Deviation Score of length of children with congenital CMV with no sequelae (—) and neurological sequelae (---) compared with the mean of controls.

FIG 6.

If CMV infection results in neurological sequelae, then the infants show reduction in height at birth, 9, 18 and 24 months. The results at three and 36 months are not significant but the confidence limits at these ages are very wide. Again it will be of value to repeat this analysis at a later date with more complete data.

It does appear, therefore, that CMV, which results in neurological sequelae, also causes growth failure and microcephaly. However, when no neurological sequelae result, there is no effect on head circumference. As yet, it is not clear if CMV, which does not result in neurological sequelae, has an effect on somatic growth, but the results suggest that there may be a progressive effect.

8. PROGNOSIS OF CHILDREN WITH CONGENITAL CMV.

The outcome of all the children in the study and the findings in the neonatal period are summarised in Table 22. Overall 12% of children with congenital CMV had a specific neurological defect on follow-up, two (4%) spastic quadriplegia, three (6%) significant bilateral sensorineural deafness (ie. requiring hearing aids), and one (2%) optic atrophy. Reduced intellectual ability without specific abnormality does not appear to be related to congenital CMV, as shown by the Griffiths testing. To assess the early features of congenital infection which may predict handicap, those with neurological handicap, excluding psychomotor delay, were compared with those without neurological defects.

None of the maternal characteristics examined appeared to be related to handicap in CMV children (Table 23). Neurological sequelae followed infection in all three trimesters of pregnancy and also recurrent maternal infection (table 24).

Children symptomatic in the neonatal period, and of lower head circumference at birth, had a poor prognosis ($p=0.03$ and 0.0002 respectively) (Table 25). When children with symptoms at birth and reduced head circumference are taken together a good prediction of subsequent handicap is possible when the criterion used to assess reduced head size >3 SDS below the mean ($p=0.0002$). All children with neurological abnormality were identified at or before

TABLE 22.

Clinical manifestations at birth and at subsequent follow-up of children with congenital CMV and controls.

	Assessment at Birth					Follow-up Assessment						
	No.	Bwt ≤3%	OFC ≤3%	CNS Defects	Hepato- megaly	Resp Symp	Other*	Spast- icity	Visual Handicap	Deafness - Sensorineural	Hepato- spleno- megaly	Pneu- moritis
Abnormal at birth	3	+	+	+	-	-	-	+	+	-	-	-
		-	-	-	-	+	+	-	-	-	+	-
		-	-	+	+	-	-	-	-	xx	-	-
No specific abnormalities at birth but permanent neurological sequelae	4	+	+	-	-	-	-	+	-	xx	-	-
		-	-	-	-	-	-	-	-	x	-	-
		+	+	-	-	-	-	-	-	x	-	-
		-	-	-	-	-	-	-	-	xx	-	-
SUB TOTAL	7	3	3	2	1	1	1	2	1	5	1	0
Normal at birth but without neurological sequelae	43	2	8	0	0	0	0	0	0	0	1	6
TOTAL Congenital CMV	50	5 10%	11 22%	2 4%	1 2%	1 2%	1 2%	2	1	5/47+	2	6
Controls	97	3 3%	11 11%	0	0	0	8	0	0	0/88+	1	0

* See text

† Number with audiological assessment

x Unilateral sensorineural deafness

xx Bilateral sensorineural deafness

TABLE 23.

Outcome in congenital CMV in relation to maternal characteristics.

		n.	No sequelae	Neurological sequelae
Maternal age:	<20	16	12	4
	20-24	17	17	0
	25+	17	15	2
Race	Asian	4	3	1
	White	32	29	3
	Black	14	12	2
Social class:	Non-manual	23	19	4
	Manual	11	10	1
	Other	16	15	1
Parity	Para 0	32	26	6
	Para 1+	18	18	0
Marital Status:	Single	23	18	5
	Married/ Cohabiting	27	26	1
Total		50	44	6

TABLE 24.

Outcome of congenital CMV in relation to type and gestation of maternal CMV infection.

Maternal infection	Gestation weeks	n.(%)	Neurological sequelae n.(%)
Primary	<16	9(18)	1(11)xx
	16-27	7(14)	1(14)x
	>27	14(28)	1(7)+
	NK	2(4)	0
Recurrent		10(20)	2(20)+ xx, xx
Not Classifiable		8(16)	1(12)x
Total		50(100)	6(100)

+ :spasticquadriplegia

x :unilateral sensorineurot deafness

xx :bilateral " "

TABLE 25.

Outcome of congenital CMV in relation to features noted at birth.

	No sequelae	Neurological sequelae	p.
Symptomatic	1	2	0.03
Asymptomatic	43	4	
Mean SDS HC (SD)	-0.49(1.35)	-2.69(2.08)	0.0002
Mean SDS Weight (SD)	-0.07(1.2)	-1.29(2.09)	0.2>0.1
Symptomatic+/- SDS HC >-3.0	1	4	0.0002
Asymptomatic+/- SDS HC <-3.0	43	2	
Total	44	6	

SDS Standard deviation
score

SD Standard deviation

HC Head circumference

the nine month assessment and hearing test. This suggests that normality at this age is an excellent prognostic feature though the number of children seen in the older age groups is still not substantial.

Virological Measures

The persistence of viral excretion was not helpful in predicting handicap since most children continued to excrete CMV in the urine at least up to 24 months (Table 26). The measurement of CMV specific IgM in cord blood samples, when available, was also compared with both subsequent handicap and the timing of maternal infection (Table 27). Unfortunately, not all the samples were tested in the same assay, so are not strictly comparable. However, the pattern suggests that high IgM titres of >3000 are more frequent in primary infections after 28 weeks gestation and low titres of <1600 are common before 28 weeks. There was no consistent relationship with neurological sequelae (table 28).

9. COMPARISON BETWEEN CHILDREN WITH CONGENITAL CMV AND THE STUDY POPULATION.

The estimated number in the study population and the number of children with congenital CMV in each group is shown in Table 29. The estimated prevalence of congenital CMV in the study population is shown by maternal age, race, marital status, parity, social class and infant sex. Maternal age, marital status, race, social class and parity were all associated with an increased prevalence of congenital CMV. To explore the independent effect of each of these maternal characteristics, the prevalence of congenital CMV was calculated for each, taking account of the other variables. For this purpose, mothers of 25 years and over were grouped together as the prevalence rates in the older age bands were the same. When analysed by maternal age, the prevalence amongst blacks born in the U.K., Africa, and the West Indies were similar, so all three origins were analysed together. Asian mothers (ie. of Indian sub-continent origin) were excluded as the number in this category was too small to be further sub-divided.

The maternal characteristics with the strongest effect on the rate of congenital CMV were age, race and marital status. Once

TABLE 26.

Outcome of congenital CMV in relation to persistence of viral
excretion: number of samples collected (% positive).

Age - months	3	9	18	24	36
No sequelae	52(90)	32(84)	21(71)	12(75)	12(58)
Neurological sequelae	4(100)	4(75)	3(66)	1(100)	1(0)
Total	56(91)	36(83)	24(70)	14(71)	13(54)

TABLE 27.

CMV specific IgM titre in relation to gestation and type of
maternal CMV infection.

Titre	Primary infection: weeks gestation			Recurrent infection
	<16	16-27	>27	
<50	0	2	0	0
100-800	0	1	0	1
800-<1600	1	2	1	0
1600-<3000	0	0	1	0
3000-<6400	0	0	1	1
6400	0	0	3	0
Total	1	6	6	2

TABLE 28.

Outcome of congenital CMV in relation to CMV specific IgM titre
in cord blood samples.

Titre	Neurological sequelae	No sequelae
<50	0	3
100 - 800	0	3
800-<1600	1	5
1600-<3000	0	2
3000-<6400	1	1
6400	1	2
Total	3	16

TABLE 29.

The estimated prevalence of congenital CMV infection by maternal characteristics and infant sex.

	Estimated population n.=23652	Congenital CMV n.=50	Rate/1000
<u>Race</u>			
Asian	2230	4	1.79
White	18525	32	1.73
Black	2099	14	6.67
<u>Marital status</u>			
single	3107	23	7.40
Married/cohabiting	20471	27	1.32
<u>Age</u>			
<20	1863	16	8.59
20-24	5268	17	3.23
25-29	7731	8	1.03
30+	8790	9	1.02
<u>Parity</u>			
Para 0	11872	32	2.70
Para 1+	11771	18	1.53
<u>Social Class</u>			
Non-manual	12901	22	1.70
Manual	8128	12	1.48
Other	2549	16	6.28
<u>Infant sex</u>			
Male	11984	26	2.17
Female	11646	24	2.06

these were accounted for, parity had no appreciable effect except that in 20-24 year old married white mothers the prevalence of congenital infection among multiparous women was 7/1280 compared with 1/1824 in nulliparous women. In view of the number of possible comparisons this could be a chance finding.

In Table 29 mothers in the "other" social class group show a higher rate of congenital CMV than mothers in either manual or non-manual social classes. Social class was assessed by paternal occupation, or maternal occupation in single mothers. The "other" group included students, the unemployed and single mothers with no definable social class. Amongst white mothers, within each age group, the rate of congenital CMV was similar in all three social groups. However, amongst black mothers, although there does not appear to be an effect of social class, when age is accounted for, the numbers in some subgroups were too small to completely exclude an effect.

The prevalence of congenital CMV by maternal race, marital status and age is shown in Table 30. Since these factors are closely interrelated, these data were subjected to grouped logistic regression to assess the independent contribution of each factor. The results are shown in Table 31. All three maternal characteristics have a significant effect on the rate of congenital CMV even after adjustment for the other factors. There were no significant interactions between these factors: so, for example, the effect of marital status is constant for all ages and races. The risk of a black mother giving birth to a child with congenital CMV is estimated to be 2.1 times that of white mothers. Similarly single mothers have an increased risk of 2.5 compared with married/cohabiting mothers, and mothers under 20 years an increased risk of nearly four times that of mothers over 25 years. These risks multiply such that a young single black mother has a risk approximately 20 times the risk for a married white woman over 25 years.

The above data includes all infants with congenital CMV and does not distinguish between those born following primary and recurrent maternal infections. It was possible to define the type of maternal infection in 42 cases (Table 32). When analysed by race and age the numbers were too small to demonstrate a

TABLE 30.

Congenital CMV by maternal race, marital status and age;
rate per 1000 live births.

Age	14-19		20-24		25+	
	CMV/n. (rate) screened		CMV/n. (rate) screened		CMV/n. (rate) screened	
White						
Single	5/656	7.6	5/665	7.8	1/855	1.2
Married/ cohabiting	0/610	0.0	8/3102	2.7	13/12720	1.0
Black						
Single	8/312	25.6	2/301	6.5	1/249	4.0
Married/ cohabiting	1/53	8.9	1/362	2.8	1/799	1.3

TABLE 31.

Risk of congenital CMV related to maternal age, race
and marital status

<u>Age:</u> (p<0.01)	Relative risk (95% C.I.)
25+ years (reference)	1
20-24 years	1.5 (0.7 - 3.3)
14-19 years	3.9 (1.8 - 9.1)
 <u>Race:</u> (p<0.05)	
White (reference)	1
Black	2.1 (1.1 -4.1)
 <u>Marital status:</u> (p<0.02)	
Married/cohabiting (reference)	1
Single	2.5 (1.3 - 5.1)

TABLE 32.

Type of maternal CMV infection resulting in congenital infection:
relation to maternal age and race.

Age		14-19	20-24	25+	Total(%)
White:	Primary	5	9	11	25
	Recurrent	0	1	2	3 (11)
Black:	Primary	6	1	0	7
	Recurrent	2	2	1	5 (42)
Asian:	Primary	0	0	0	0
	Recurrent	0	1	1	2 (100)
All races:	Primary	11	10	11	32
	Recurrent	2	4	4	10 (24)

significant pattern but recurrent infections are most frequent in older women and non-caucasians.

The frequency of previous exposure to CMV infection in the screened population was available in 8059 women and the data presented in fig.7. The prevalence of CF antibody was consistent in the Asian mothers at all ages. In both black and caucasian mothers the seropositivity rate rose considerably with age. In black mothers from 68% in women under 20 years to 86% in women over 30 years. In caucasian women the rates rose from 38% to 52% respectively.

10. THE TRANSMISSION OF CMV INFECTION IN FAMILIES OF CHILDREN WITH CMV INFECTION.

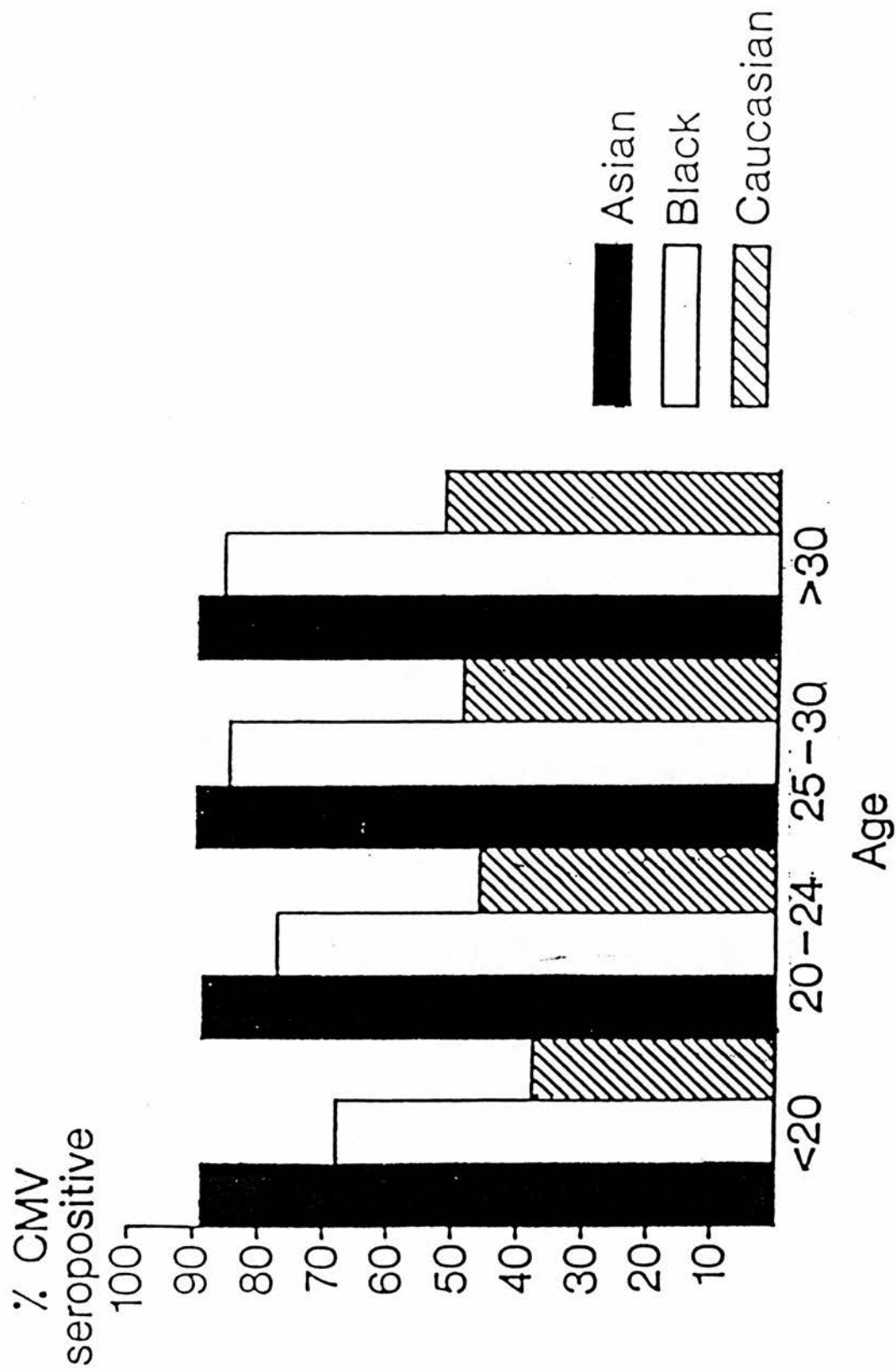
Eight family groups have been studied, seven with a congenitally infected infant and one where the infant acquired CMV while in hospital (figs.8,9).

Families 1-4 and 7 consisted of both parents and an older sibling in each case. In families 1,3,4, and 7 the mother had experienced a primary infection during pregnancy. In the remaining family (2) the mother experienced a presumed recurrent infection, being CMV CF antibody positive at 11 weeks gestation with no demonstrable CMV specific IgM antibody.

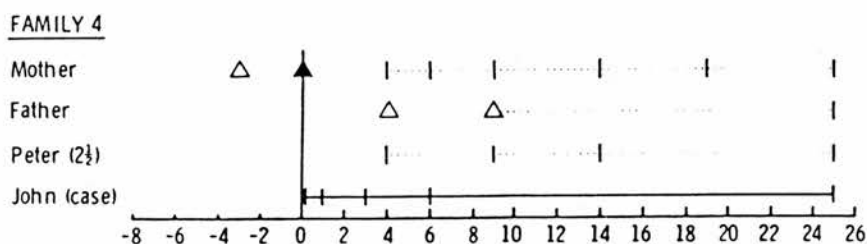
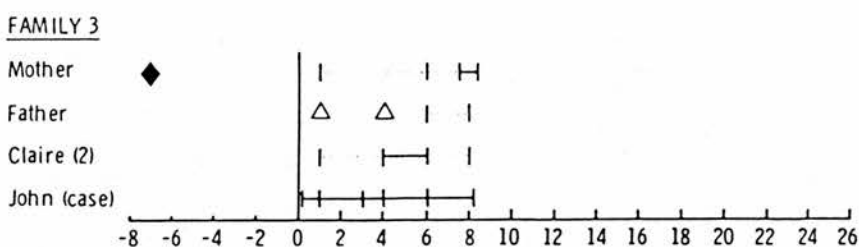
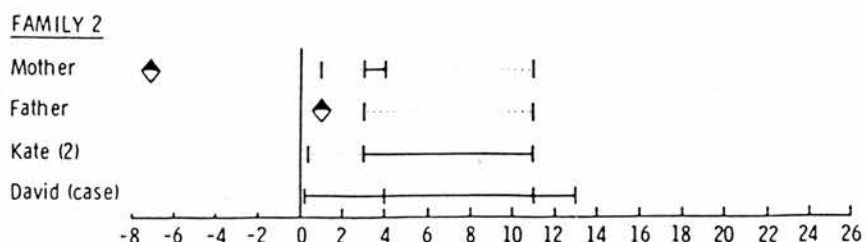
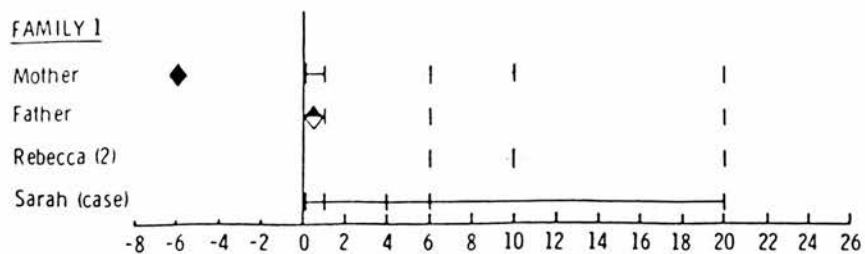
Families 5 and 6 consist of parents and congenitally infected child only. In each case the mother had experienced a primary CMV infection.

Viral excretion by mothers of congenitally infected infants.

In six of the seven families virus was isolated from mothers of congenitally infected infants. In three from urine only and in the remaining three from breast milk in addition to urine. The period of maternal virus excretion was short in each case. In family 6 endonuclease typing has shown no dissimilarity in the viruses isolated from mother and baby confirming vertical transmission (fig.10). However, in family 7, virus isolated from mother's breast milk, whilst showing an identical pattern with enzyme Hind III, shows minor genetic variation with enzyme Bgl III (fig.11, marks a,b). This suggests a minor genetic change as a result of passage to a different host.



CMV antibody status of 8057 women at their first antenatal attendance.



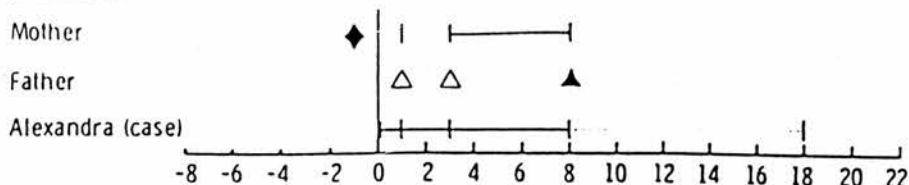
Age of case in months.

Pattern of CMV infection in families with a recognised source of CMV (case).

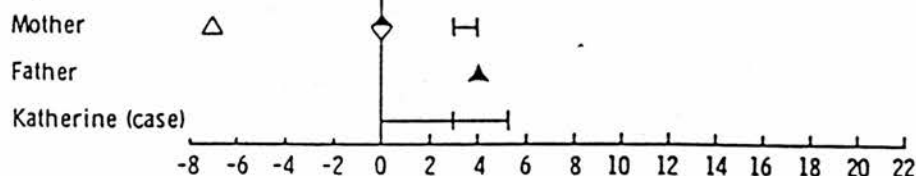
FIG 8.

key: CMV CF-ve: △
 CMV CF+ve: ▲
 CMV IgM-ve: ▽
 CMV IgM+ve: ▼
 Urine collected for viral culture: I
 Viral excretion: —

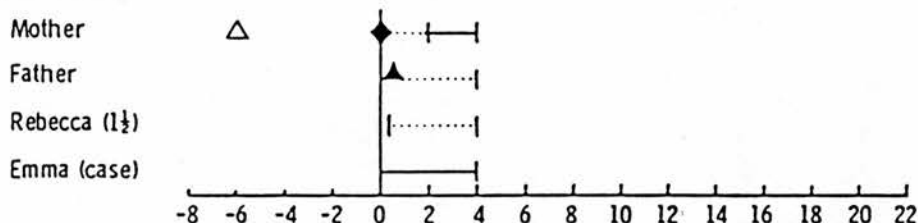
FAMILY 5



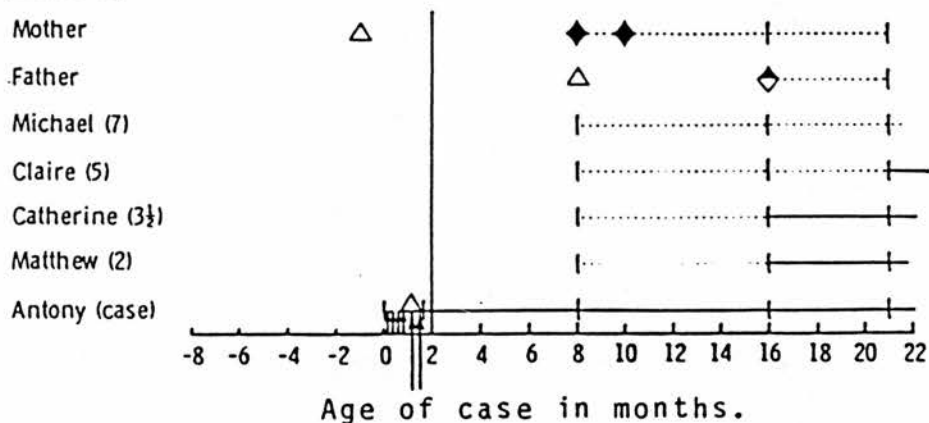
FAMILY 6



FAMILY 7










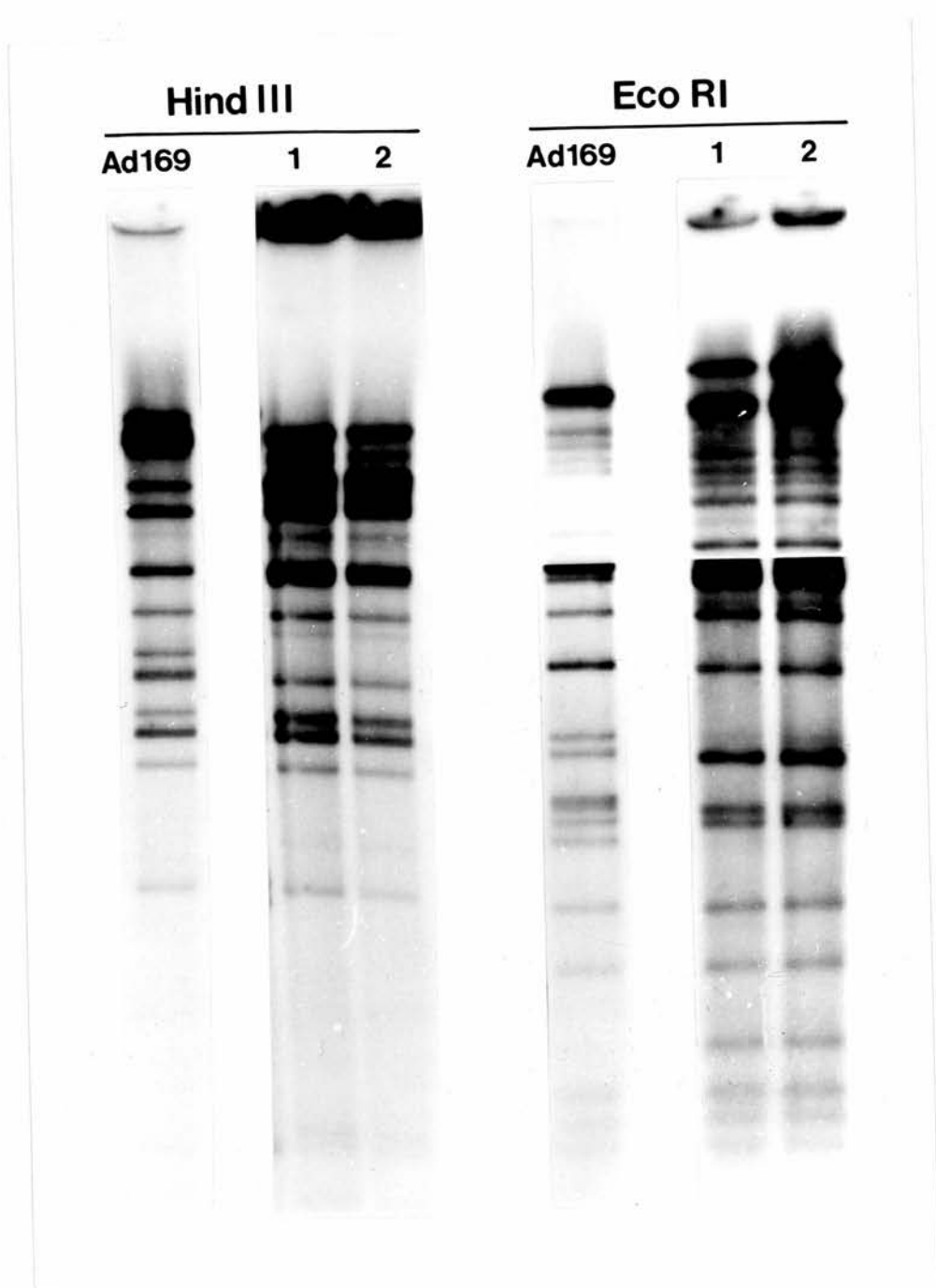
FAMILY 8



Pattern of CMV infection in families with a recognised source of CMV (case).

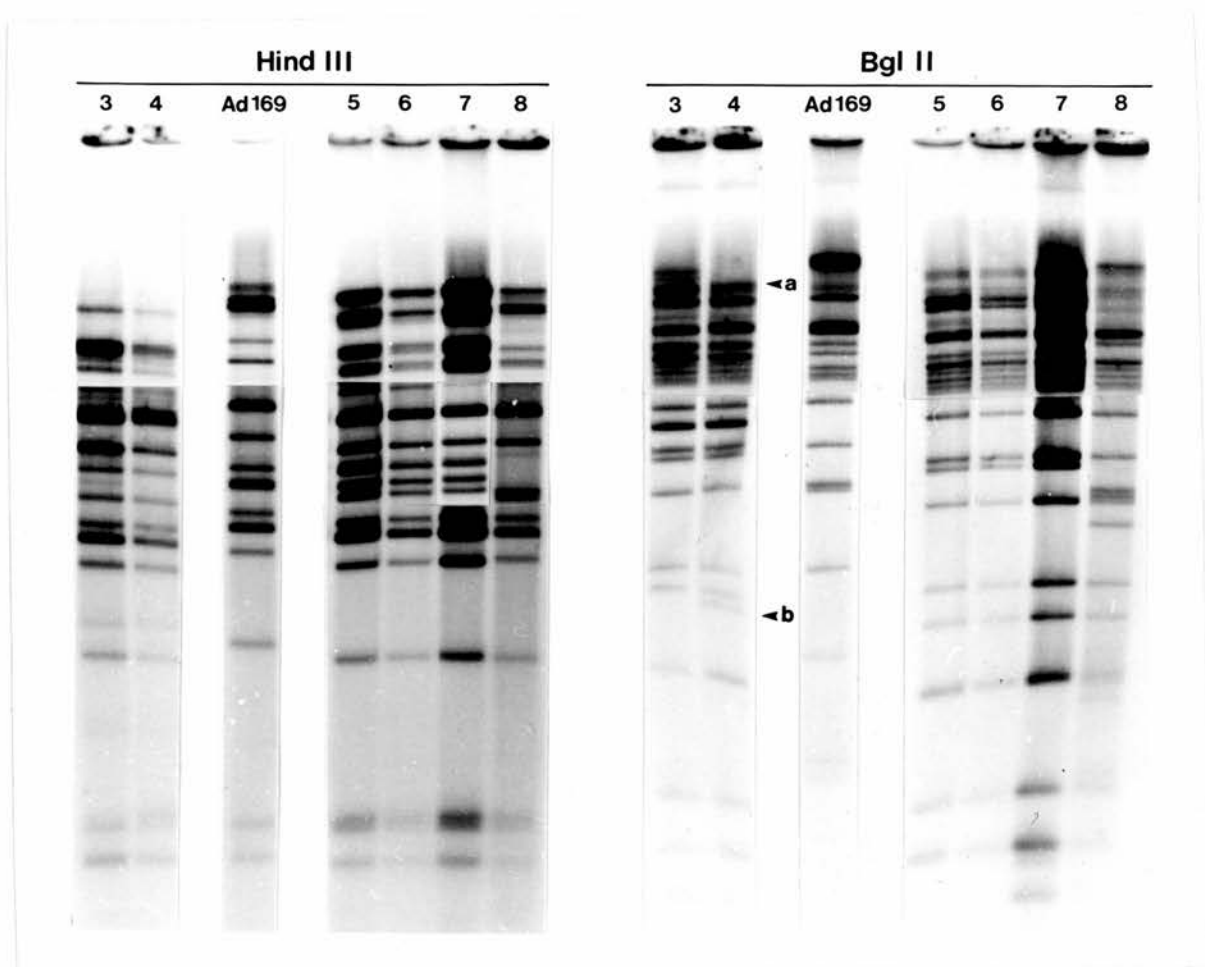
FIG 9.

key: CMV CF-ve: 
 CMV CF+ve: 
 CMV IgM-ve: 
 CMV IgM+ve: 
 Urine collected for viral culture: I 
 Viral excretion: 
 Blood transfusion: 



Oligonucleotide patterns of CMV DNA from mother and infant of family 6.
DNA was digested with enzymes Hind III and EcoRI:
Lane 1 - virus from infant's urine
Lane 2 - virus from mother's urine

FIG 10.



Oligonucleotide patterns of CMV DNA from families 7 and 8.
DNA was digested with enzymes Hind III and EcoRI:

Family 7 :Lane 3 - virus from infant's urine
 Lane 4 - virus from mother's breast milk
 Family 8 :Lane 5,6,7 - virus from 3 siblings
 Lane 8 - virus from infant's urine

FIG 11.

Acquisition of CMV by fathers.

In four families, (1, 2, 6 & 7) the father was CMV antibody positive when first tested after the birth of the congenitally infected infant but virus was not isolated and it is not possible to confirm the source of infection. In families 3 and 4 the father did not acquire CMV during the period of study (eight and 25 months respectively.) Both were exposed to their congenitally infected sons excreting high titres of virus continuously for the study period and partners who had experienced a recent primary infection.

One father, in family 5, sero-converted 16-36 weeks after the birth of a congenitally infected daughter. Unfortunately specimens for viral culture could not be obtained so it is not possible to define an intrafamily source of CMV.

Acquisition of CMV by siblings.

None of the five siblings studied were positive on virus culture at the time of the birth of the congenitally infected sibling. However, two (in families 2 & 3) acquired CMV after three and four months exposure respectively. The other three were negative after four, 18, and 25 months exposure respectively. DNA endonuclease typing of isolates of the two contacts demonstrate a nondissimilar genetic structure compared with the virus isolates of their congenitally infected siblings, suggesting intrafamily transmission had occurred, probably from the younger infant, but spread from the mother cannot be excluded.

CMV infection following hospital acquired CMV.

Family 8 provided an opportunity to study a family with only one known source of CMV infection. The family consisted of parents and four children. The fifth child (Antony) was delivered at 30 weeks gestation following severe rhesus isoimmunisation. Two intrauterine transfusions were required before birth, three exchange transfusions within 48 hours of birth and three further transfusions to treat anaemia at 20, 32 and 42 days. The last three transfusions were donated by fit hospital staff and the donors of the last two transfusions were known to possess CMV CF antibodies. Congenital CMV was excluded as part of the prospective study and CMV CF titre was <5 at 30 days. CMV was isolated at 60 days from urine suggesting acquisition from one of

the last two blood transfusions. Despite this he was asymptomatic and discharged at 67 days. He continues to excrete high titres of CMV in urine after 20 months.

His mother seroconverted within the first five months since his discharge and his father between five and 13 months. (The absence of CMV specific IgM suggests primary infection occurred five to nine months after discharge.) Unfortunately virus was not isolated from either parent to confirm the source of infection. During the period of study three of four siblings have excreted CMV. The viruses of all three siblings were demonstrated by DNA endonuclease typing to be genetically not dissimilar but distinct from the virus excreted by Antony suggesting a source infection independent of the index case (fig.11).

DISCUSSION

This study is the first large prospective study of congenital CMV in Britain. Only the Collaborative Study 127 has studied similar numbers of children with congenital CMV from a British population. However, the nature of that study was not prospective, and so the results are difficult to apply to other populations. The present study provides the prospective data to estimate the effects of congenital CMV on a British population. Using these data, decisions may be made on the advisability of screening for CMV in pregnancy and screening neonates. In addition the need for preventive measures, such as vaccination, may be assessed. The final results of the population study 153, from which this cohort of congenitally infected infants was derived, will allow rates of primary CMV infection in pregnancy, rates of congenital infection, and frequency of intrauterine transmission to be calculated.

The rate of permanent neurological sequelae in infants with congenital CMV, in this study, was at least 8-12%. This includes 10% with sensorineural deafness, 4% with spastic quadriplegia and 2% with visual handicaps. These results are similar to other prospective studies in USA, 83,103,167 Canada 175 and Sweden 1 despite the wide variation in the prevalence of congenital infection throughout the world.

THE EFFECT OF DIAGNOSTIC METHODS ON THE RESULTS.

It has been shown that the method of diagnosis of congenital infection may have profound effects on the prevalence rates and type of congenitally infected infant selected. 4,83,131,167 The diagnostic method used here, virus isolation from a throat swab, was the only feasible method of screening such a large population. This method has a false negative rate of 12%. To reduce the effect of this, urine samples were collected in the infants of mothers who seroconverted prior to delivery. Some infants with

congenital infection will, however, not have been included in the study as the throat swab was falsely negative. Infants of mothers who were seropositive at booking following early primary infection or maternal reactivation will thus be under-represented by 10-15%. When combined with the seroconversion group the overall rate of false negative results would be 8% in the study as a whole.

It is noteworthy, however, that occasional problems in virus culture from urine samples mean that false negative results may occur with this method. Screening, using samples of cord sera, to detect CMV specific IgM, is the least satisfactory method, as illustrated by the two studies on the same population. 131 The only advantage of screening by this method, is that large numbers of samples may be collected and stored for analysis in batches later, or retrieved for testing in children found to have sensorineural deafness or other neurological problems in later life, when virus isolation cannot distinguish between congenital and acquired infection. 166

It is felt, therefore, that the results of this study may be compared with other prospective studies despite differences in the diagnostic method. 1,83,167,175,205

THE USE OF CONTROLS TO ASSESS THE EFFECT OF CONGENITAL CMV.

Controls were selected in all but two cases. Two controls were selected in each case, to guard against the possibility of loss of contact. In the three years of the study, only six controls have lost contact after varying follow-up periods. The controls were adequately matched for infant sex, maternal race and age. In many cases, matching on the second line criterion was possible and no differences were found between cases and controls. However, to reduce the variability in some analysis it was appropriate to use regression techniques. In this form of analysis allowance can be made for differences in background characteristics so that the full effect of CMV on the variable, such as developmental quotient or growth, could be assessed. While this method did not detect any significant differences not apparent on inspection, it allows a more confident prediction of the effect of each variable. Thus, for example, it can be seen

that the mean DQ of a child from manual social class is 6 points below the expected mean of children of non-manual social class. The effect of CMV with neurological sequelae is, however, much more significant, reducing the mean DQ by 35.6 points. By this method, then, it can be confidently stated that, in this example, congenital CMV not associated with neurological sequelae does not significantly effect intellect, and so counter claims that congenital CMV may account for a significant number of children with mild mental handicap, dyslexia and minimal brain damage who previously had no diagnostic label. 84 Similar arguments explain the need to use this type of analysis of data on growth so that the effect of socioeconomic factors may be distinguished from the effect of congenital CMV.

ANTENATAL PROBLEMS ASSOCIATED WITH CMV.

No increase in antenatal problems was noted in the mothers of children with congenital CMV. None had symptomatic infection suggestive of maternal CMV. Infants with congenital infection, who subsequently went on to develop normally, did not suffer any retardation of intrauterine growth. It is possible that some fetal loss may occur as a result of maternal primary CMV 72 but there is no evidence from this or other studies that premature labour is precipitated by CMV. 205 It has been suggested that infants with CMV may be of lower birth weight. 205 This is so in infants who subsequently develop neurological sequelae, who are frequently symptomatic at birth, 146 but there appears to be a clear distinction between this group and those who subsequently develop normally, rather than continuous effect on birth weight. SFGA infants were not significantly more frequent amongst infants with congenital CMV compared with controls and it is of little value screening SFGA infants for CMV. 163 Similar findings have been reported in rubella. 115 Severely affected infants are, however, often small, and, in particular, have microcephaly. 146,232 Congenital abnormalities occurred no more frequently than expected and it is probable that earlier reports of an association were due to chance occurrences. 15,122,125,205 There is little evidence of a teratogenic role of CMV infections in pregnancy.

INFANTS SYMPTOMATIC IN THE NEONATAL PERIOD.

Three infants were ill in the neonatal period and in two congenital infection was suspected. One suffered extreme growth retardation and dystonia as a neonate, the other hypotonia and hepatomegaly. Both developed significant handicaps on follow-up. One further child had pneumonitis, retrospectively attributed to CMV. Thus 6% had neonatal symptoms and two thirds subsequently developed neurological handicaps. These findings are in accord with most other studies. 113,131,146,205,232 No child in this study, however, presented with hepatosplenomegaly, purpura, and thrombocytopenia; the commonest combination of presenting features described in cytomegalic inclusion disease. 125,146 Two children, however, developed hepatosplenomegaly in the first three months and, as described by McCracken et al, 125 this persisted for up to eighteen months in one child, followed by complete recovery. Later presentations of this type have only previously been noted by Starr et al. 205 Since congenital infection was not suspected in either case, these features may have been missed had the children not been followed prospectively.

RESPIRATORY COMPLICATIONS OF CONGENITAL CMV.

The role of CMV as a respiratory pathogen is only now being recognised. No other prospective study has described pneumonitis as a problem in congenital CMV. This complication occurred in 12% of infants in this study. It is recognised that organisms such as CMV, chlamydia and pneumocystis may give rise to opportunist infections in the first few months of life. 198 These often present, as in this study, at 3-4 months, when passively acquired maternal antibody levels start to wane. Double infections are common, as in one child in this study with coincident chlamydial infection. All of the infections in this study were mild, and would not routinely have been investigated. This may be why pneumonitis has not previously been reported in congenital CMV after the neonatal period.

CMV pneumonitis is not always benign and severe infections may occur, particularly in infants with other problems such as broncho-pulmonary dysplasia. 229 It is possible to treat severe cases with high titre CMV specific immunoglobulin, so correct diagnosis is important. 186

MAJOR NEUROLOGICAL HANDICAPS RESULTING FROM CONGENITAL CMV

In the present study 2 children (4%) developed spastic quadriplegia, a rate similar to other prospective studies. (2-6.6%) 4,83,166,175,205 As would be expected from previous studies 195 one child, with cerebral palsy, also suffered from optic atrophy (2%). No other specific neurological complications have been noted as yet, other than sensorineural deafness, but it is possible that minor neurological handicaps such as those described by Williamson et al 232 may become apparent in the older children. The number of children who have reached the three-year examination is still small as yet but no evidence of specific problems such as language disorders are so far apparent. In the Griffiths testing all subscores gave similar results to the total score and the Stott test demonstrated equal coordination abilities in congenitally infected infants and controls.

Sensorineural deafness is the commonest neurological sequelae of congenital CMV, the rate in this study being 10%; 6% were significant bilateral (ie. hearing loss of 60 dB in the better ear.) All of these children require hearing aids and will require special educational help. Previous studies reported rates of deafness of up to 50% of cases of congenital CMV. 167 However, when analysed in more detail it is apparent that some of the figures were elevated due to the diagnostic method and very wide definition of "deafness". When the same population was restudied by Stagno et al, 199 with the use of the more sensitive screening test for congenital CMV (isolation of virus from urine samples), the rate of deafness was found to be 7.7%, similar to that reported here. However, it is not possible to be confident that all cases of unilateral deafness have been identified as yet, since very few children in this study were mature enough to have a full audiometric assessment with the use of headphones and masked bone

conduction. The two reported with unilateral deafness were diagnosed by distraction testing, and require confirmation later. Finally the possibility of progressive deafness requires further study. 36,232 Other prospective studies have not reported progressive deafness and Saigal et al 175 tested most of their subjects twice, at 3 and 5 years, with no significant change in results. It is therefore mandatory to retest all children in this study at 5 years.

The effect of CMV on intelligence is still not clear. For some years it has been suggested that CMV is a major cause of mental handicap. 48,84,131 CMV certainly causes severe mental retardation in conjunction with major neurological handicaps such as spastic quadriplegia as in two cases here. However, only one prospective study has shown a significant effect of CMV on IQ. 83 Indeed, in that study social class had a marked effect, and it is possible that there was an additive effect. Also several children with other neurological handicaps which may have affected their performance were not excluded. The regression analysis of Griffith testing in the present study demonstrates that social class certainly has an effect on DQ which is significant and independent from the presence or absence of CMV. There was no interaction between these variables. These data, combined with the negative findings of Saigal et al and Kumar et al and the equivocal findings of Reynolds et al do not suggest any effect of CMV on intelligence, when neurological disabilities are allowed for. 107,167,175 Indeed, the pathological findings in congenital CMV are mainly specific, localised lesions, which are likely to produce specific effects, inner ear involvement causing sensorineural deafness, periventricular foci causing upper motor neurone effects, and chorioretinitis and optic atrophy causing blindness. These features are distinct from the toxic effects of poisons such as alcohol and lead, 174,246 both of which cause dose related effects with mild to moderate reduction in overall intellectual abilities, often in the absence of specific neurological features. This is illustrated by one congenitally infected child in the present study, who also suffered from fetal alcohol syndrome. She is now over three years old, and below the third percentile for height, weight and head circumference with

mild mental retardation, but there is no specific neurological defect. It is possible that the major pathology in this child is fetal alcohol syndrome, and that congenital CMV is incidental.

THE EFFECT OF CONGENITAL CMV ON GROWTH

Infants with symptomatic congenital CMV are often growth retarded 146 and microcephaly is a poor prognostic sign at birth. In the present study children who subsequently developed neurological sequelae were significantly shorter, of reduced birth weight and head circumference. There was no evidence of subsequent catch-up growth in these children. In addition, it has been demonstrated that a head circumference of more than 3 SD below the mean carries a poor prognosis.

Since the intellectual progress of infants with congenital CMV, but no subsequent sequelae, is similar to controls, it is not surprising that birth weight and head circumference are also similar to controls. The height of these children, however, did not match the other parameters. There was no difference in birth length and length at three months, but mean height gradually fell below the expected mean at 9, 18, and 24 months, the difference becoming significant at 18 and 24 months. This was an unexpected finding and has not been noted in congenital CMV before. The full cohort have not yet reached the older age groups so the results are by no means confirmed and indeed the trend is reversed in the fifteen children measured at 36 months. This suggested effect of congenital CMV does not match the pattern of CMV defects described up to now. It is extremely important to follow this group sequentially and reanalyse the data when they reach five years.

THE EFFECT OF TYPES OF MATERNAL CMV INFECTION.

It was possible in this study to establish the type of maternal infection in all but 16% of cases. This compares favourably with Ahlfors et al 5 who was unable to classify the type of maternal infection in 42% of infants with congenital CMV. The use of the RIA to detect CMV specific IgM, in association with recent primary infection, was crucial in improving the specificity

of the test. The demonstration of IgM by this method in 86% of primary infections within 16 weeks of seroconversion and absence after 16 weeks 98 means that the presence or absence of CMV specific IgM can be used to classify a large group of women who present for antenatal care with CMV CF antibodies detectable, but no previous samples available to determine when seroconversion occurred. 74 This test has now been applied to screening in pregnancy for early primary infection as well as its retrospective use demonstrated here. 76

Taking into account the problems of screening, using the collection of throat swabs and the false negatives of the IgM assay, the relative contribution of primary infections was 76% and recurrent infection 24%. There was no significant difference in the rate of congenital CMV following primary maternal infection in each trimester, (I-30%, II-23%, III-46%) and the rate of primary infection is probably constant throughout pregnancy. 76

It is not possible to estimate the stage of pregnancy at which women experience reactivation of CMV and transmit infection to the fetus. Even if CMV is cultured from cervix or urine this does not mean that the fetus is infected at the time of positive virus culture, as transmission may result from local extension of infection in the cervix or endometrium, and not from viraemia. Thus it is possible to assess the effect of infection at each stage of pregnancy following maternal primary infection but not following recurrent maternal infection. However, there is no evidence from this study that gestation of primary infection has an effect on outcome. The rates of subsequent handicap are similar following maternal infection in all three trimesters and confirms the findings of Stagno et al. 201 Ahlfors et al 6 did note more symptomatic infections following first trimester infections but this data was drawn from collaborative work, where only one child was identified in her prospective study, and the rest were referred presumably because of clinical suspicion of intrauterine infection.

The small number of prospective studies, 5,201 which have been able to define the type of maternal infection, does mean that it is not yet possible to be confident of the role of maternal reactivation in relation to subsequent outcome in congenital CMV. There are, however, four case reports of handicap following

confirmed maternal reactivation. 3,87,173,239 Two infants with sensorineural deafness, one with additional spastic quadriplegia, in the present study were born following presumed maternal reactivation. Unfortunately, since the RIA test of CMV specific IgM is only 86% sensitive 98 it is not possible to exclude the possibility that both these mothers may have experienced early primary infection with an absent or unusually transient rise in IgM. The rate of handicap, however, is similar in all studies, despite wide variations in the population screened, with wide ranges of seropositivity in the maternal population. 25 This suggests, irrespective of the proportion of congenital CMV arising from maternal primary infection and reactivation, the outcome is similar.

OVERALL EFFECTS OF CONGENITAL CMV ON A BRITISH POPULATION

Children with congenital CMV in this study can be divided into two separate groups. The majority, ie. 44 of the 50 children (88%) have suffered no significant permanent sequelae resulting from congenital CMV infection. The remainder (12%) have longterm sequelae, unilateral deafness in 4% and major handicaps such as spastic quadriplegia, bilateral sensorineural deafness in 8%. Only two early features were associated with eventual poor outcome, symptoms of intrauterine infection in the neonatal period or head circumference more than 3 SD below the expected mean. Other features previously associated with poor outcome, type of maternal infection, 201 gestation of primary infection, 6,136 high titre of CMV specific IgM in cord blood, 75 were not confirmed. Indeed it may be that the IgM titre, in cord blood, is more commonly a reflection of the time since primary maternal infection than a measure of severity. The titre of virus excretion, in the urine, has also been associated with a poor prognosis, 75 but this measure of viral activity was not available in the present study. However, the majority of infants in both groups excreted virus consistently for at least two years, by which time all neurologically affected children had been identified clinically.

While most children, who developed problems later, could have been identified at birth, one child with sensorineural deafness was

apparently entirely normal up to the age of six months. It is not possible, therefore, to be entirely confident that all those who will have problems later may be identified at birth.

It is possible now to predict the impact that congenital CMV may have on a British population. Assuming a birth rate of 650,000 per year, in England and Wales, and a rate of congenital CMV of 3-4/1000 live births, 127,153 1950-2600 infants will be born with congenital CMV per year in Britain. Assuming 10% have appreciable deafness, neurological defects, or both, 195-260 infants will be born each year with serious handicaps. If a further 5% have minor handicaps such as unilateral deafness, the total number of children affected will be 3-400. Hence the number of children with serious handicaps attributable to cytomegalovirus is comparable to that of children born with congenital rubella defects in a non-epidemic year, prior to the vaccination era. 46

SCREENING FOR CMV INFECTION IN PREGNANCY AND NEONATES

It has been suggested that the best method of control of congenital CMV is to screen in pregnancy for primary infection and then to terminate the pregnancies affected. 10 This advice was based on a number of assumptions of CMV in pregnancy. Firstly, that reactivation was not associated with congenital infection and secondly, that as in rubella, early CMV was more likely to cause serious handicaps than infections in later pregnancy. This advice is no longer tenable.

In an audit of their screening programme of primary CMV infection in pregnancy, Griffiths and Babbonian 76 have demonstrated that, since at least 50% of primary infections are not transmitted in utero and a large number of infections cannot be diagnosed early enough to allow termination to occur, many uninfected infants and congenitally infected infants who will not develop sequelae, will be lost, but a significant number of congenitally infected infants who will subsequently be handicapped will be missed should such a policy be instituted. In the present study the most seriously handicapped infant was born following a maternal primary infection diagnosed by the presence of CMV specific IgM at 28 weeks gestation when the mother first presented

for antenatal care. Reviewing the data in this study in retrospect, only four cases of seroconversion were identified before 28 weeks gestation and the infants of all four mothers have asymptomatic congenital infection. The other mothers experiencing seroconversion before 28 weeks gestation were only diagnosed in retrospect.

In addition to the 18 infants born following seroconversion, the mothers of the remaining 63% of congenitally infected infants were seropositive at booking and at least 20% were born following a maternal reactivation. At present, there is no serological method of diagnosis in maternal reactivation, as the antibody response to recurrent infection has not yet been clearly defined. Virus isolation in seropositive women represents evidence of reactivation but may bear no temporal relationship to when, or if, the infant may acquire infection in utero. Thus, screening in pregnancy will also be ineffective in identifying all women at risk of delivering a congenitally infected child.

It has been demonstrated here that screening of neonates is practical but since there is no treatment available it is debatable whether this is justifiable. It will allow the identification of a group at risk of developing sensorineural deafness, but 80-90% of these infants will develop normally. If there is an effective screening service to identify early problems with hearing or psychomotor development then those with problems will be offered prompt treatment without requiring prior identification of CMV. In addition, screening for CMV will subject the parents of the majority 80-90% of congenitally infected infants to at least a year of anxiety until they can be sure their child will not suffer as a result of this infection. Therefore, unless a safe, effective antiviral preparation can be produced, there is little justification in screening for CMV in pregnancy or among neonates. It may, however, be of value to store neonatal blood samples to aid in the diagnostic screening of children who present later with neurological handicaps. Congenital CMV may account for up to 20% of all cases of sensorineural deafness and positive diagnosis of viral etiology can allay fears in parents that future children are likely to suffer from genetically determined deafness.

THE PREVALENCE OF CONGENITAL INFECTION IN SUBSECTIONS OF THE POPULATION

The results in this study confirm the prime importance of maternal age in determining the prevalence of congenital CMV 113,127,201,205 and the lesser effect marital status. 113,127 It also highlights the increased prevalence of congenital CMV in the infants of black women, which is predictable from the study of primary CMV by Griffiths et al. 77 None of these studies used regression techniques to exclude correlations between variables. This may explain why other suggested associations such as social class 83 and parity 113,205 are not confirmed here. Reduced parity is intimately linked with younger maternal age and it has been shown here that the determining factor is age rather than parity. Indeed, there is some evidence that increased parity may increase the risk of acquisition of primary CMV in mothers of children attending a day nursery. 150

The association of social class described by Hanshaw et al 83 may be a reflection of the method of social stratification 90 using educational criteria as well as profession. Thus most young single mothers will be in the lower social class group. These mothers are unclassified under the Registrar General's classification 165 and are defined here as "other" social class. However, the increased prevalence in this group is accounted for in the regression analysis by maternal age, race, and marital status.

The prevalence of congenital CMV depends on a complex inter-relation of several factors. The rate of primary maternal CMV and the rate of intrauterine transmission will vary depending on the population studied. Similarly, the rate of maternal reactivation is also very variable and we still have no measure of rate of intrauterine transmission in this situation. Thus the rate of congenital infection in a particular community will depend on the age, race and marital status of the pregnant population, the rate of previous maternal CMV infection, and the rate of primary infection. An expression of both of these is the seropositivity rates in the community. The number of seropositive women at each age determines the number of women at risk of reactivation of

previous infection and the rate of rise of seropositivity is an expression of the rate of primary infection.

In the present study, the seropositivity rate rose rapidly in younger women, particularly between the under 20 years and the 20-25 years groups. Thus it may be inferred that the rate of primary infection is higher in younger women. The rate of primary infections is higher in black women compared to white as they have a greater chance of contact with a seropositive partner. The rise in seropositivity rates slows in the older age groups so fewer women are experiencing primary infections. This may be because older women have fewer contacts, despite the increased possibility that the contacts she has are more likely to be seropositive.

The commonest method of spread of CMV, in adults, has not yet been defined. There is an abrupt rise in seropositivity rates in the late teenage years, a rise not seen in nuns. 39 This suggests that intimate contact, either oral or sexual, is required for transmission and the number of partners may be relevant. Thus the social behaviour of young women may determine the rate of acquisition of CMV, and different social behaviour patterns in subsections of the population may, in part, account for the different rates between single and married women, and between racial groups.

Reactivation of maternal infection is closely linked with recent primary infection but may also represent reinfection from a different source. 101 Since the seropositivity rate among black mothers is higher than whites, it is not surprising that congenital infection is significantly more frequent following maternal reactivation in black women, compared with whites. Several studies have suggested a direct link between seropositivity and congenital infection rates. This is true when comparing Ivory Coast 180 and Chile 202 with Britain 208 and Canada. 113 Also within a community comparing low socioeconomic class blacks and middle class whites in Alabama.

However, there are significant exceptions. Firstly, in Japan, where a universally high seropositivity rate is matched by a low rate of congenital infection. 95 A similar finding in this study is the low prevalence of congenital CMV in infants of Asian mothers in association with a 90% seropositivity rate. All

congenital infections in the Asian population and those studied in Japan resulted from maternal reactivation. Thus the 10% of seronegative women in those communities rarely seroconvert during the childbearing years, despite contact with a population who are 90% seropositive. Secondly, the rate of reactivation is not high in the Asian population, compared with lower socioeconomic class blacks in the Southern USA. 201 This suggests that factors other than seropositivity determine the rate of reactivation, such as nutrition, immune competence, or perhaps the likelihood of direct exposure to active infection with resultant reinfection. The effect of affluence on a highly seroimmune population requires further study to determine the frequency of reactivation of CMV infection.

These findings are important for two reasons, firstly predictions of the number of infants born with CMV will require modification to account for the race, age and marital status of the population as a whole. There is no evidence that the infants of different groups are selectively more likely to develop sequelae as a result of congenital CMV, so areas with high concentrations of young black mothers may have a very high rate of congenital deafness resulting from congenital CMV. This is analogous to the concentration of rubella defects in the children of West Indian immigrants in Britain in past epidemics. Secondly, control measures such as vaccination will be ineffective if they are developed purely to prevent primary maternal infection. It may be that acquisition of infection in childhood may be more protective against intrauterine transmission in later pregnancy in an affluent society.

INTRAFAMILY SPREAD OF CMV INFECTIONS

DNA endonuclease typing confirms the finding of Huang et al 91 that congenital infection in infants is acquired from mothers, although minor genetic differences did appear in one mother/child pair, suggesting minor genetic adaptation to the new host. These differences however are quite distinct from major differences seen in non-epidemiologically related strains. The DNA patterns are unique to each epidemiologically related strain with no overlapping

yet reported.

The study of fathers in families with a congenitally infected infant cannot confirm if infection was acquired from the infant or the mother, but in two families the fathers remained seronegative after 8 and 24 months respectively. This shows that transmission is not inevitable despite the presence in the family of at least two potential sources.

In studying siblings, it was thought unethical to subject children to blood samples to assess their previous exposure to CMV, thus when virus was isolated it was not possible to determine when primary infection occurred. Typing confirms intrafamily transmission either from mother or congenitally infected sibling. It is possible, however, that the sibling may have acquired infection outside the home, then transmitted virus to the mother. Since children with acquired CMV excrete virus for prolonged periods, 192 and both infants were CMV negative initially, it is probable that infection was transmitted from mother or congenitally infected child to older sibling. The inevitability of intrafamily infection may not be assumed, however, as shown in family 8, where multiple sources were responsible for family infection.

These family studies illustrate the low infectivity of CMV except in situations of prolonged contact and would argue against a significant risk of acquisition by professionals such as nurses and teachers. The results of studies of the risk of acquisition of CMV, in a professional situation, using primary infection rates and seropositivity are equivocal. One such case (6) was investigated in the present study and occupational acquisition was excluded. The only situation of major risk is a primary care giver who is seronegative caring for an infected child at home. This will arise if a congenitally infected child goes into foster care. It will also occur when a premature infant of a seronegative mother acquires CMV by blood transfusion, following which there is a very high maternal seroconversion rate. 242 In other situations, routine hygiene methods should be sufficient to prevent transmission. 244

PROBLEMS WHICH REQUIRE FURTHER STUDY

This study has defined the major consequences of congenital CMV in a British population. Several problems require further study. The present cohort requires follow-up for some years to confirm that no other major handicaps come to light, and that those normal, so far, remain entirely normal. Further study is also required to define the exact rate of minor hearing defects, visual handicaps, and exclude effects of congenital infection on higher intellectual function, such as intelligence, specific language defects, learning disorders and behaviour. The growth of otherwise asymptomatic children requires further study to define the importance of the preliminary effect on growth shown here.

It will be some time before sufficient children have been followed up in this and other studies to confirm that type and time of congenital infection have no effect on subsequent handicaps. A study in an area of high prevalence of congenital CMV, resulting from maternal reactivation, may be required to study this aspect. The frequency of reactivation and the precipitating factors are still little understood particularly in relation to different rates in different populations such as the Asian families in Britain, Japan, and the black population of Alabama, USA. The effect of improved affluence and other socioeconomic changes may become evident as more second generation Asian women start to have families in Britain.

It will be clear from this thesis that the biological relationship between man and CMV is very complex and only by detailed study of the epidemiology of CMV infection is it possible to understand this relationship and plan methods of control of infection and the prevention of significant neurological handicap in the childhood population.

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APPENDICES.

CHARING CROSS CMV STUDY

SURNAME _____

FIRST NAMES _____

HOSPITAL NO. _____

E.D.D. _____

BLOOD COLLECTION DATE _____

day month year

Date of birth 9-14

First day of last menstrual period 15-20

(Put 99 for day if not precisely remembered)

Previous pregnancies

Number of livebirths ☐ 21

Number of stillbirths ☐ 22

Total number of previous pregnancies over 28 weeks 23-24

Number of miscarriages ☐ 25

Number of terminations ☐ 26

Date of conclusion of last pregnancy (including miscarriages and terminations) month year

Marital Status (tick relevant boxes)

27

single married divorced separated widowed co-habiting
☐ ☐ ☐ ☐ ☐ ☐

Race

28

(for mixed race either tick two boxes or specify under 'other')

Asian Caucasian Negro Oriental Other (specify)
☐ ☐ ☐ ☐ _____

Country of birth _____ 29-30

Occupation

For those in paid work please give 1) type of business or industry 2) actual work done 3) level of responsibility.

For those not in paid work please state whether 1) never worked 2) student 3) housewife, or 4) unemployed at present in which case please state 'unemployed' and then give details of last job as above.

Woman's occupation before home duties _____

31-32

Date left work month year or still working ☐ 33-36

Husband or partner's occupation: (If no steady partner please put 'no partner') _____

37-38

Maternal details form.

APP 1.

CMV STUDY – INFANT'S DETAILS

Please complete this form at the infant's first possible examination, preferably within the first 48 hours of life, and send with a throat swab to the laboratory

Date of Birth:

Is this infant:

singleton

twin 1

twin 2

other (specify)

☐
☐
☐

_____ 9

Sex: _____ 10

Birth Weight: _____ grams 11–14

Number of weeks gestation: _____ 15–16

Is this by dates ☐, ultrasound ☐, or clinical assessment ☐

(tick one) 17

E.D.D.

Mother's Name: _____

Hospital No: _____

Use Mother's hospital sticker if available

Has infant shown any obvious malformations or problems at birth?

_____ 18–19

Does the infant require special care? Yes ☐ No ☐ 20

Date swab was taken 21–26

Infant details form.

APP 2.

STUDY SERIAL NO: CHARING CROSS CMV STUDY
MATERNAL MASTER-CARD

(Maternal antenatal form to be stuck on here)

Card 1: <input type="text"/> 1	Serial No: <input type="text"/>	2-8	Card 2: <input type="text"/> 2	Serial No: <input type="text"/>	2-8		
<input type="text"/>	9-14	<input type="text"/>	15-20	<input type="text"/>	21		
<input type="text"/> 22	<input type="text"/> 23-24	<input type="text"/> 25	<input type="text"/> 26	<input type="text"/> 27	<input type="text"/> 28		
<input type="text"/>	29-30	<input type="text"/>	31-32	<input type="text"/>	33-36	<input type="text"/>	37-38
Lab No: <input type="text"/>							
Blood 1 (up to 27 weeks gestation)			Blood 3 (36 weeks to term)				
Collection date	<input type="text"/>	39-44	Collection date	<input type="text"/>	9-14		
Date of test	<input type="text"/>	45-50	Date of test	<input type="text"/>	15-20		
CMV CFT titre	<input type="text"/>	51-54	CMV CFT titre	<input type="text"/>	21-24		
CMV IF titre	<input type="text"/>	55	CMV IF titre	<input type="text"/>	25		
Blood 2 (28 to 35 weeks)			CMV IgM				
Collection date	<input type="text"/>	56-61	Collection date	<input type="text"/>	26-31		
Date of test	<input type="text"/>	62-67	Date of test	<input type="text"/>	32-37		
CMV CFT titre	<input type="text"/>	68-71	CMV IgM	<input type="text"/>	38-41		
CMV IF titre	<input type="text"/>	72	Rubella				
			Collection date	<input type="text"/>	42-47		
			Date of test	<input type="text"/>	48-53		
			Rubella HAI titre	<input type="text"/>	54-57		
			Outcome of pregnancy				
			Date of delivery	<input type="text"/>	58-63		
			Outcome	<input type="text"/>	64		

(1 = livebirth; 2 = stillbirth; 3 = neonatal death; 4 = spontaneous abortion; 5 = delivered elsewhere; 9 = no information)

Maternal Master Card.

APP 3.

CONGENITAL CMV FOLLOW-UP

Name:

Case no.

--	--	--

Address:

Study no:

.....

NHS no:

..... Tel:

CXH no:

GP

.....

..... Tel:

HV

.....

..... Tel:

	D.O.B.	6/52	3/12	9/12	AUDIO	18/12	2 yrs				
Date											
Virology											
Exam.											

PROBLEMS

- 1.
- 2.
- 3.
- 4.
- 5.
- 6.

Infant Record.

APP 4.

HISTORY

Child's Name _____ Case no.

--	--	--	--

 9.11

MOTHER

FATHER

Name _____ Name _____

Age (at child's birth) _____ Age (at child's birth) _____

Race _____ Race _____ 12

Country of birth _____ Country of birth _____ 13

Previous history:

Dm Fits ↑BP Other Dm Fits ↑BP Other

☐ 14 ☐ 15 ☐ 16 ☐ 18 ☐ 19 ☐ 20

17
21

Family history:

Deafness? Psychomotor retardation Atopy Deafness? Psychomotor retardation Atopy

☐ 22 ☐ 23 ☐ 24 ☐ 26 ☐ 27 ☐ 28

Other: _____

25

Mother's history: this pregnancy

Smokes? ☐ 30 BP \geq 90D and/or proteinuria ☐ 31 APH ☐ 32 Dm ☐ 33

Other (1) _____

(2) _____ 3

Abroad during this pregnancy? If so, where? _____ 36-37
and for how long? _____ weeks

Presentation: (tick one) Cephalic Breech Other (specify)

Onset of Labour: Spontaneous Induced
(tick one) ☐ ☒ 39

Card

Study Number

Case No. 28

			9.11
--	--	--	------

12

13

14 15 16 17

18 19 20 21

☐ 22 ☐ 23 ☐ 24

25

☐ 26 ☐ 27 ☐ 28

29

☐ 30 ☐ 31 ☐ 32 ☐ 33

34

39

36.37

38

39

Delivery (tick one)

Normal

☐

Forceps high

☐

Forceps low

☐

Forceps rotation

☐

LSCS elective

☐

LSCS emergency

☐

Ventouse

☐

40

☐

40

Indication:

Mother's CMV status — cases only

1. Primary infection/seroconversion

☐

2. Presumed primary infection (seropositive at booking, IgM positive)

☐

3. Known reactivation (seropositive specimen *before* this pregnancy)

☐

4. Presumed reactivation (seropositive at booking, IgM negative < 12 weeks)

☐

5. Undefined (seropositive at booking, IgM negative > 12 weeks)

☐

9. No information

☐

41

☐

41

NEONATAL DETAILS

Child's name _____ Case no:

OFC at birth mm 42-44 Length at birth mm 45-47

Apgar 48-49 at 1 minute, 50-51 at 5 minutes Placental weight gms 52-54

Resuscitation

O₂ by face mask ⁵⁵ for ⁵⁶⁻⁵⁸ mins

O₂ ambu " mins

IPPV " mins

Drugs ⁵⁹ Specify _____

Respiratory problems

RDS/TTN > 24 hours ⁶⁰

Meconium aspirate ⁶¹

Pneumonia ⁶²

Other _____ ⁶³

Jaundice? ⁶⁴ Max. Bilirubin μ mol/l 65-67 Hepatosplenomegaly? ⁶⁸

CNS problems

Fits ⁶⁹

CNS infection ⁷⁰

Other ⁷¹ _____

Congenital malformations

CVS ⁷²

LS ⁷⁶

Resp. ⁷³

Genitourinary ⁷⁷

Abdo. ⁷⁴

Other _____ ⁷⁸

CNS ⁷⁵

Other problems

Was this child admitted to SCBU? ⁸⁰ date _____

Neonatal diagnosis on discharge

Has he/she been followed up by hospital? _____ If so, consultant's name: _____

INITIAL EXAMINATION

Child's name _____ Case no: 9-11

Gestation _____ Age _____ Date: _____ 12-17

General appearance? _____

OFC 18-20

Eyes: Squint ☐

Cataract ☐

Other _____ 21

Rash ☐ 22

Chest examination ☐ 23

Heart: any abnormal sounds or murmurs? ☐ 24

Femoral pulses ☐ 25

Abdomen: Liver ☐ 26

Spleen ☐ 27

Other _____ 28

Genitalia ☐ 29

Skeletal abnormalities ☐ 30

CNS examination: state? _____

Fix follow ☐ Moro ☐

Light reflex ☐ Stepping ☐

Rooting ☐ Movement symmetrical ☐

Grasp ☐ Tone symmetrical ☐ 31

Conclusions: _____

32

Urine

Collected on

Innoculated on

Result ☐ 33

Baby's breastmilk

Collected on

Innoculated on

Result ☐ 34

Card ☐ 1

Study number

2-8

Case no. 9-11

12-1

18-20

☐ 21

☐ 22

☐ 23

☐ 24

☐ 25

☐ 26

☐ 27

☐ 28

☐ 29

☐ 30

☐ 31

☐ 32

☐ 33

☐ 34

ASSESSMENT AT 3, 9 AND 18 MONTHS

Name _____ Case no: 9-11

Age _____ Date of examination _____ 12-17

General health

Has the child been ill since last visit?

Severity Treatment

Otitis media 18 19

URTI 20 21

LRTI 22 23

Other 24 Specify

Do the parents think the child's

Hearing is normal ? abnormal ref. 25

Vision is normal ? abnormal ref. 26

Development

Progress since last seen?

Developmental assessment

Passive posture 27-28

Active posture 29-30

Gross movement 31-32

Manipulation 33-34

Vision 35-36

Hearing 37-38

Speech 39-40

Social/interactive 41-42

Self-care 43-44

Conclusion: normal ? abnormal 45

Physical examination

Height 46-49

Weight 50-53

OFC 54-56

Physical state: normal ? abnormal 57

Urine: collected on _____ innoculated _____ result 58

Tracheal aspirate collected on _____ innoculated _____ result 59

Card 1

Study number 2-8

Case no. 9-1
12-

18 19

20 21

22 23

24

25

26

27-28

29-30

31-32

33-34

35-36

37-38

39-40

41-42

43-44

45

46-49

50-53

54-56

57

58

59

DEVELOPMENT ASSESSMENT

Field 1: POSTURE AND LARGE MOVEMENTS

a) Passive posture

1) Supine Position

Head to one side, knees apart	0
Head in midline	1
Lifts legs into vertical and grasps feet	2

2) Ventral Suspension

Head droops below plane of body, hips flexed, limbs hang down	0
Head in line with body, hips semi-extended	1
Head above line of body, hips and shoulders extended	2

3) Pull to sit

Marked head lag	0
Considerable head lag, when body vertical head held momentarily erect before falling forwards	1
Little or no head lag	2
Braces shoulders and pulls self up	3

4) Sitting Position (Supported)

Back completely curved	0
Back fairly curved, intermittently holds head up	1
Back straight except in lumbar region, head mostly held up	2
Back straight	3

STYCAR developmental record form.

APP 5.

(2)

b) Active posture

5) Prone Position

Head sideways, hips and limbs extended	0
Head sideways resting on cheeks, buttocks high with knees flexed under abdomen, arms close to chest with elbows flexed	1
Head to side, limbs flexed, elbows away from body, buttocks moderately high	2
Head to side, chin raised intermittently, moderate extension of hips	3
Head and upper chest held up on forearms for prolonged periods, buttocks flat	4
Head and chest well up, limbs stretched out in 'swimming' position	5
Supports weight on flattened palms and extended arms, looks around alertly	6
Gets into crawling position	7

6) Sitting Position

Not applicable 0

Sits alone momentarily, supports body on hands	1
Sits alone momentarily, without support	2
Sits alone for prolonged periods	3
Leans forwards or wideways to reach toy without losing balance	4
Gets into sitting position from prone or supine	5

7) Rolling

N/A 0

Rolls prone to supine	1
Rolls supine to prone	2

8) Standing

Held standing sags at knees and bears on weight on feet	0
Held standing bears some weight on feet	1
Held standing takes full weight on feet	2
Stands holding on	3
Pulls self to stand	4

(3)

c) Gross movements

9) Locomotion

N/A

00

Placing and stepping reflexes present	
Progresses by rolling or squirming	01
Attempts to crawl (maybe backwards)	02
Crawls on hands and knees or shuffles on buttocks	03
Walks with hands held	04
Walks around furniture or pushing wheeled toy	05
Walks alone, feet wide apart, arms up for balance	06
Feet only slightly apart, can turn corners and stop suddenly	07
Runs, cannot continue round obstacles	08
Picks up object from floor without falling	09
Runs confidently, stopping and starting with care and avoiding obstacles	10
Jumps on both feet from low step	11
Can walk on tiptoe	12
Stands on one foot, runs on tiptoe	13
Skips, hops	14

10) Stairs

Crawls upstairs and may slither down backwards	1
Creeps backwards downstairs or bumps down on buttocks facing forwards	2
Walks upstairs with hand held, two feet to a step	3
Walks up and down stairs confidently, two feet to a step	4
Walks alone upstairs, one foot to a step, and down two feet to a step	5
Walks alone up and down stairs, one foot to a step	6

Field 2: VISION AND FINE MOVEMENTS

a) Manipulation

11) Hands

Hands loosely open	00
Hands closed and thumb turned in	01
Hand regard and finger play	02
Clasps hands and presses palms together	03
Palmar grasp using whole hand	04
Passes toy from one hand to the other	05
Holds two cubes, one in each hand and brings them together	06
Picks up small object between finger and thumb with 'inferior' pincer grasp	07
Neat pincer grasp between finger and thumb	08
Throws toys to the floor deliberately	09
Turns pages of a book, several at a time	10
Turns pages one at a time	11
Threads beads	12
Cuts with scissors	13

12) Bricks

N/A 0

Tower of 2	1
Tower of 3	2
Tower of 4-6	3
Tower of 7+	4
Copies bridge	5
6 cube steps	6
10 cube steps	7

13) Drawing

N/A 0

To and fro scribble	1
Circular scribble	2
Imitates vertical and/or horizontal line	3
Imitates circle	4
Imitates cross	5
Draws man with head	6
Draws man with head, legs and trunk	7
Draws house	8
Copies square	9
Copies triangle	10

14) Holds pencil in palmar grasp	1
Holds pencil between thumb and fingers	2

(5)

b) Vision

15) Visual function

No visual response	0
Turns towards diffuse light	1
Briefly follows torch light at 30 cm. distance	2
Watches a face or dangling object in line of vision and follows with eyes less than 90°	3
Fixates on object and follows by turning head up to 90°	4
Follows dangling object through 180°	5

16) Visual comprehension N/A 0

Fixates on small objects and reaches out to grasp them	1
Watches falling toy and then forgets it	2
Looks in correct place for fallen toy	3
Watches movements of people at distance or out of window with interest	4
Shows interest in pictures	5
Enjoys simple picture books and recognises some items	6
Recognises fine detail in picture books	7
Matches two primary colours. Describes action from picture	8
Matches and names four colours	9

(6)

Field 3: HEARING AND SPEECH

a) Hearing

17) Auditory function

No auditory response	0
Startled by sudden noise	1
Responds to voice or gentle sound	2
Looks towards sound of mother's voice	3

18) Verbal comprehension N/A 0

Turns head towards sound source	1
Attentive to everyday sounds	2
Recognises own name	3
Hands over common objects on request	4
Understands simple phrases or questions	5
Obeys simple instruction	6

(7)

b) Speech

19) Vocalisation

Makes no sounds	0
Makes occasional grunting sounds	1
Vocalises when pleased	2
Laughs, chuckles and squeals in play	3
Babbles continuously and shouts to attract attention	4
Imitates adult's playful sounds, e.g. cough, b'rr and smacking lips	5

20) Language N/A 00

Incessant jargon containing most vowels and many consonants	01
One word with meaning	02
Several words with meaning	03
Attempts to join in nursery rhyme	04
Puts two or more words together to form simple sentences	05
Names familiar objects and pictures	06
Knows full name	07
Uses interrogative pronouns 'What?', 'Who?' and personal pronouns 'I, Me, You', correctly	08
Able to carry on simple conversation and describe events	09
Tells stories with confabulation - big and small	10
Knows and repeats several nursery rhymes	11
Knows name and address, age and birthday	12

Field 4: SOCIAL BEHAVIOUR AND PLAY

a) Interactive Behaviour

24) Social Behaviour

Sleeps when not being fed or handled	00
Smiles to social stimulation	01
Responds with chuckles and excited movements to friendly handling	02
Shy with strangers	03
Shows annoyance when frustrated	04
Plays 'pat-a-cake' and/or waves bye-bye	05
Restless and curious regarding people, objects and events in immediate surroundings	06
Imitates simple everyday activities	07
Intensely curious regarding environment, little comprehension of common dangers	08
Resistive and rebellious when thwarted	09
Plays with other children but will not share toys	10
Understands sharing playthings and adults' attention	11
Understands conception of present, past and future	12
Takes turns and shares	13
Chooses own friends	14

25) Play

No interactive play	00
When offered rattle, reaches for it and shakes to make it sound	01
Plays 'peek-a-boo'	02
Finds toy which is partially but not wholly hidden	03
Puts bricks in and out of container when shown	04
Quickly finds hidden toy	05
Explores properties and possibilities of toys and other objects with interest	06
Plays contentedly alone near familiar person	07
Pushes and pulls large toys skilfully	08
Kicks large ball	09
Throws small ball overhand. Catches large ball	10
Rides tricycle well	11
Climbs trees, ladders. Make believe play	12
Plays ball games with rules	13

b) Personal self-care behaviour21) Feeding

No active response to bottle or spoon feeding	00
Puts hand to bottle when feeding	01
Tries to grasp spoon	02
Holds, bites and chews a biscuit	03
Drinks from a cup with assistance	04
Holds spoon and brings it to mouth but cannot prevent it turning over	05
Holds cup with both hands and drinks without too much spilling	06
Holds spoon and gets food safely to mouth	07
Lifts cup, drinks and replaces it on table without difficulty	08
Eats skilfully with spoon	09
Eats with fork and spoon	10
Eats with knife and fork	11

22) Dressing

Passive acceptance of dressing routine	0
Holds up arms when being dressed or undressed	1
Helps constructively with dressing by holding out arms and legs appropriately	2
Takes off shoes, socks or hat	3
Puts on hat and shoes	4
Pulls down pants or knickers	5
Can pull up pants or knickers	6
Can dress and undress, but not buttons and laces	7
Can manage buttons and laces	8

23) Toilet

In nappies. No awareness of wetting/soiling	0
Indicates wet or soiled pants	1
Indicates toilet needs by restlessness or vocalisation	2
Has bowel control	3
Dry by day	4
Verbalises toilet needs in reasonable time	5
Usually dry at night if lifted	6
Washes hands	7
Brushes teeth	8
Washes hands and face	9

Additional items for older children

- | | | |
|-------------------------|---|--------|
| 1. Heel Toe standing | <input type="checkbox"/> | 60 |
| 2. Jump and clap | <input type="checkbox"/> | 61 |
| 3. Matchsticks | <input type="checkbox"/> | 62 |
| 4. Pinboard L & R hands | <div style="display: flex; align-items: center;"> <div style="text-align: center; margin-right: 5px;">R</div> <div style="border: 1px solid black; width: 40px; height: 20px; display: flex; align-items: center; justify-content: center;"> </div> <div style="text-align: center; margin-left: 5px;">L</div> </div> | 63; 64 |
| 5. Bridge of bricks | <input type="checkbox"/> | 65 |
| 6. Formboard | <input type="checkbox"/> | 66 |

Scoring	No attempt	1
	Attempt	2
	Good try	3
	Succeed	4
	Time Limit	5.

- | | | | |
|---------------------|-----------|-----|-----------------------------|
| 7. Draw a man below | Scribble | = 1 | <input type="checkbox"/> 67 |
| | Head +1 | = 2 | |
| | Head +3-4 | = 3 | |
| | Head +5-6 | = 4 | |
| | Head +7 | = 5 | |

Tests of coordination.

APP 6

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APP 7.